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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
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EDWARDS)
)
For: BLEOMYCIN GENE CLUSTER)
COMPONENTS AND THEIR USES)
) San Francisco, California

Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

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PATENT APPLICATION TRANSMITTAL

Sir:

Transmitted herewith for filing is the patent application of inventor(s) Ben Shen, Liangcheng Du, Cesar Sanchez, Mei Chen and Daniel J. Edwards, for "BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES." Enclosed are:

1. 83 pages of the specification, including 73 claims and an abstract.
2. 12 sheets of drawings.
3. 56 pages of Sequence Listing.
4. An oath or declaration of the inventors (unsigned).

The filing fee is being deferred at this time.

Dated: January 5, 2000.

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U.S. Patent Application For

BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES

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BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit under 35 U.S.C. §119 of provisional applications USSN 60/115,435, filed on January 6, 1999, and USSN 60/118,848, filed on February 5, 1999, both of which are herein incorporated by reference in their entirety for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This work was supported in part by an Institutional Research Grant from the American Cancer Society and the School of Medicien, University of California, Davis, National Institutes of Health Grant Number A140475, and a grant from the Searle Scholars Program of the Chicago Community Trust. The Government of the United States of America may have certain rights in this invention.

FIELD OF THE INVENTION

This invention relates the field of polyketide synthesis and nonribosomal polypeptide synthesis. In particular this invention pertains to the isolation of the bleomycin gene cluster which encodes the first identified hybrid polyketide synthase/nonribosomal peptide synthetase pathway.

BACKGROUND OF THE INVENTION

Polyketides and nonribosomal peptides are two large families of natural products that include many clinically valuable drugs, such as erythromycin and vancomycin (antibacterial), FK506 and cyclosporin (immunosuppresant), and epothilone and bleomycin (BLM) (antitumor). The biosyntheses of polyketides and nonribosomal peptides are catalyzed by polyketide synthases (PKSs) (Hopwood (1997) *Chem. Rev.* 97: 2465; Katz (1997) *Chem. Rev.,* 97: 2557; C. Khosla, (1997) *Chem. Rev.,* 97: 2577; Ikeda and Omura, (1997) *Chem. Rev.,* 97: 2591; Staunton and Wilkinson(1997) *Chem. Rev.,* 97: 2611; Cane *et al.*(1998) *Science* 282: 63) and nonribosomal peptide synthetases (NRPSs) (Cane *et al.*(1998) *Science* 282: 63. Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675), respectively. Remarkably, PKSs and NRPSs use a very similar strategy for the assembly of these two distinct classes of natural products by sequential condensation of short carboxylic acids and amino acids, respectively, and utilize the same 4'-phosphopantetheine prosthetic group, via a thioester linkage, to channel the growing polyketide or peptide intermediate during the elongation processes.

Both type I PKSs and NRPSs are multifunctional proteins that are organized into modules. (A module is defined as a set of distinctive domains that encode all the enzyme activities necessary for one cycle of polyketide or peptide chain elongation and associated modifications.) The number and order of modules and the type of domains within a module on each PKS or NRPS protein determine the structural variations of the resulting polyketide and peptide products by dictating the number, order, choice of the carboxylic acid or amino acid to be incorporated, and the modifications associated with a particular cycle of elongation. These features of PKS and NRPS inspired us to search for a hybrid PKS and NRPS system. Since the modular architecture of both PKS (Cane *et al.*(1998) *Science* 282: 63; Katz and Danadio (1993) *Ann. Rev. Microbiol.* 47: 875 (1993); Hutchinson and Fujii (1995) *Ann. Rev. Microbiol.* 49: 201) and NRPS (Cane *et al.*(1998) *Science* 282: 63, Stachelhaus *et al.* (1995) *Science* 269: 69; Stachelhaus *et al.* (198) *Mol. Gen. Genet.* 257: 308; Belshaw *et al.* (1999) *Science* 284, 486) has been exploited successfully in combinatorial biosynthesis of diverse "unnatural" natural products, it is imagined that a hybrid PKS and NRPS system, capable of incorporating both carboxylic acids and amino acids into the final products, could surely lead to even greater chemical structural diversity.

The BLMs, differing structurally at the C-terminal amines of the glycopeptides, are a family of antibiotics produced by *Streptomyces verticillus* (*Sv*). BLMs exhibit strong antitumor activity through a metal-dependent oxidative cleavage of DNA or RNA in the presence of molecular oxygen and are incorporated into current chemotherapy of several malignancies under the trade name of Blenoxane® that contains BLM A2 and BLM B2 as the principal constituents (Sikic *et al.* Eds. (1985) *Bleomycin Chemotherapy,* Academic Press, New York; Natrajan and Hecht (1994) pages 197-242 In: *Molecular Aspects of Anticancer Drug-DNA Interaction Vol. 2*, Neidle and Waring Eds., Macmillan, London). Umezawa, Fujii, Takita, and co-workers extensively studied the biosynthesis of BLM in *Sv* ATCC15003 by feeding isotope-labeled precursors and by isolating various biosynthetic intermediates and shunt metabolites, establishing that the BLMs are in fact natural hybrid metabolites of polyketide and peptide biosynthesis (Takita and Muroka (1990) pages 289-309 In: *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of ß-Lactams and Microbial Peptides*, Kleinkauf and Von Döhren Eds., W. de

Gruyter, New York). On the assumption that BLM biosynthesis follows the paradigm for peptide and polyketide biosynthesis, we predict that the Blm megasynthetase, which catalyzes the assembly of the BLM backbone from nine amino acids and one acetate, should bear the characteristics of both NRPS and PKS, providing an excellent model to study the mechanism by which NRPS and PKS could be integrated into a productive biosynthetic system to synthesize a hybrid peptide and polyketide metabolite (Fig. 1A) (Shen *et al.* (1999) *Bioorg. Chem.* 27: 155).

SUMMARY OF THE INVENTION

This invention pertains to the isolation and elucidation of the bleomycin gene cluster. Nucleic acid sequences encoding all of the open reading frames (ORFs) that encode polypeptides sufficient to direct the biosynthesis of bleomycin are provided. The nucleic acids can be used in their "native" format or recombined in a wide variety of manners to create novel synthetic pathways.

In one embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid selected from the group consisting of a nucleic acid encoding any one of Blm open reading frames (ORFs) 8 through 41, and/or a nucleic acid encoding a polypeptide encoded by any one of Blm open reading frames (ORFs) 8 through 41, and/or a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II and the nucleic acid of a bleomycin-producing organism as a template. The nucleic acid may comprise one or multiple (*e.g.* two, more preferably 3 or more) bleomycin open reading frames (*i.e. BLM* ORFs 8 through 41). One preferred nucleic acid comprises a nucleic acid encoding a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation. In another preferred embodiment the nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of blmI, blmII, and blmXI.

In another embodiment this invention provides an isolated nucleic acid encoding a (biosynthetic) module comprising two or more (more preferably three or more, most preferably four or more) catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain. Preferred nucleic acids comprises a nucleic acid encoding one or more proteins comprising a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS. Particularly preferred nucleic acids comprise an open reading frame from SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

In still another embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid encoding a protein encoded by a gene from a BLM gene cluster. Preferred nucleic acids encode a protein encoded by a gene selected from the group consisting of blmI, blmII, and blmXI. In another embodiment, preferred nucleic acids encode a protein encoded by a gene selected from the group consisting of blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX. In still yet another embodiment, the nucleic acid comprises a nucleic acid encoding a protein encoded by blmVIII. Particularly preferred nucleic acids comprise a nucleic acid selected from the group consisting of blmI, blmII, and blmXI. Other particularly preferred nucleic acids comprise a nucleic acid selected from the group consisting of blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX, while still other particularly preferred nucleic acids comprise blmVIII.

In still yet another embodiment, this invention provides an isolated nucleic acid comprising a nucleic acid that encodes a protein comprising at least one catalytic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain, and that hybridizes to a nucleic acid selected from the group consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28, orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40 under stringent conditions. In certain embodiments this also includes nucleic acids that would stringently hybridizes indicated above, but for, the degeneracy of the nucleic acid code. In other words, if silent mutations could be made in the subject sequence so that it hybridizes to he indicated sequence(s) under stringent conditions, it would be included in certain embodiments. A preferred isolated nucleic acid comprises a nucleic acid encoding a module. A particularly preferred isolated nucleic acid comprises a nucleic acid encoding a BLM gene.

This invention also provides a nucleic acid comprising a nucleic acid selected from the group consisting of consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28, orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40, or an allelic variant thereof. Preferred nucleic acids comprise a nucleic acid that is a single nucleotide polymorphism (SNP) of a nucleic acid selected from the group consisting of consisting of orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15, orf15, orf16, orf17, orf18, orf19, orf20, orf21, orf22, orf23, orf24, orf25, orf26, orf27, orf28, orf29, orf30, orf31, orf32, orf33, orf34, orf35, orf36, orf37, orf38, orf39, and orf40.

This invention also provides an isolated gene cluster comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

In one embodiment this invention provides an isolated multi-functional protein complex comprising both a polyketide synthase (PKS) and a polypeptide synthetase (NRPS) and/or an isolated nucleic acid encoding a multi-functional protein complex comprising both a polyketide synthase (PKS) and a polypeptide synthetase (NRPS).

This invention also provides various *blm* cluster polypeptides or blm cluster-derived polypeptides. Thus, in one embodiment this invention provides an isolated polypeptide comprising a catalytic domain encoded by a nucleic acid of a bleomycin gene cluster wherein said nucleic acid comprises a nucleic acid selected from the group consisting of a nucleic acid encoding any one of Blm open reading frames (ORFs) 8 through 41; and/or a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II. Preferred polypeptides comprise an enzymatic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain. Particularly preferred polypeptides are encoded by the nucleic acids described above and herein.

This invention also provides expression vectors comprising any of the nucleic acids described herein and/or host cells (*e.g.* Streptomyces) transfected and/or transformed with any of these expression vectors. A preferred host cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

This invention also provides methods of use of the *blm* and *blm*-derived nucleic acid(s) and/or polypeptides. One such method is a method of chemically modifying a biological molecule. The method involves contacting a biological molecule that is a substrate for a polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames with the polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames, whereby the polypeptide chemically modifies the biological molecule. In one particularly preferred embodiment, the biological molecule is an amino acid and said polypeptide is a peptide synthetase. In another preferred embodiment, the polypeptide is a methyl transferase. Other substrates and *blm* encoded polypeptides are illustrated in Table II.

In another embodiment this invention provides a method of coupling a first amino acid to a second amino acid. This method involves contacting the first and second amino acid with a recombinantly expressed bleomycin nonribosomal peptide synthetase (NRPS). A preferred NRPS is selected from the group consisting of NRPS-5, NRPS-4, NRPS-3, NRPS-9, NRPS-8, and NRPS-7. Another preferred NRPS is selected from the group consisting of NRPS-6, NRPS-2, NRPS-1, and NRPS-0. The contacting can be *in* vivo (*e.g.* in a host cell) or *ex vivo.*

In another embodiment this invention provides a methods of coupling a first fatty acid to a second fatty acid, said method comprising contacting the first and second fatty acids with a recombinantly expressed bleomycin polyketide synthase (PKS). Again, the contacting can be *in* vivo (*e.g.* in a host cell) or *ex vivo.*

In still another embodiment, this invention provides a method of producing a bleomycin or bleomycin analog. The method involves providing a cell transformed with an exogenous nucleic acid comprising a bleomycin gene cluster encoding polypeptides sufficient to direct the assembly of said bleomycin or bleomycin analog; culturing the cell under conditions permitting the biosynthesis of bleomycin or bleomycin analog; and isolating said bleomycin or bleomycin analog from said cell.

This invention also provides an isolated nucleic acid comprising a nucleic acid encoding a phosphopantetheinyl transferase said nucleic acid encoding a phosphopantetheinyl transferase being selected from the group consisting of: a nucleic acid encoding the protein encoded by the nucleic acid of SEQ ID NO:3; a nucleic acid amplified by polymerase chain reaction (PCR) using primers that specifically amplify ORF 41 (primers: SEQ ID NO:71 and SEQ ID NO:72) and *Streptomyces* nucleic acid as a template; a nucleic acid encoding a polypeptide having phosphopantetheinyl transferase activity where said nucleic acid specifically hybridizes to the nucleic acid of SEQ ID NO: 3 under stringent conditions. In one embodiment, the nucleic acid comprises the nucleic acid of SEQ ID NO:3.

In another embodiment, this invention provides a polypeptide comprising a phosphopantetheinyl transferase encoded by SEQ ID NO:3 or a polypeptide having phosphopantetheinyl transferase activity and the sequence encoded by the nucleic acid of SEQ ID NO: 3 or conservative substitutions of that polypeptide.

Also provided are vectors comprising a nucleic acid encoding a phosphopantetheinyl transferase (*e.g.,* as described above) and cells transfected with the vector.

This invention also provides a method of converting an apo carrier protein to a holo carrier protein, said method comprising reacting said apo-carrier protein with a recombinant phosphopantetheinyl transferase encoded by SEQ ID NO:3 and coenzyme A thereby producing a holo-carrier protein.

In certain embodiments, this invention specifically excludes one or more of open reading frames 1 through 41. In particularly preferred embodiments, this invention excludes open reading frames 1 through 7 (Orf 1- Orf 7).

DEFINITIONS

The "polyketide synthases" (PKSs) refers are multifunctional enzymes, related to fatty acid synthases (FASs). PKSs catalyze the biosynthesis of polyketides through repeated (decarboxylative) Claisen condensations between acylthioesters, usually acetyl, propionyl, malonyl or methylmalonyl. Following each condensation, they typically introduce structural variability into the product by catalyzing all, part, or none of a reductive cycle comprising a ketoreduction, dehydration, and enoylreduction on the β-keto group of the growing polyketide chain. PKSs incorporate enormous structural diversity into their products, in addition to varying the condensation cycle, by controlling the overall chain length, choice of primer and extender units and, particularly in the case of aromatic polyketides, regiospecific cyclizations of the nascent polyketide chain. After the carbon chain has grown to a length characteristic of each specific product, it is typically released from the synthase by thiolysis or acyltransfer. Thus, PKSs consist of families of enzymes which work together to produce a given polyketide. Two general classes of PKSs exist. One class, known as Type I PKSs, is represented by the PKSs for macrolides such as erythromycin. These "complex" or "modular" PKSs include assemblies of several large multifunctional proteins carrying, between them, a set of separate active sites for each step of carbon chain assembly and modification (Cortes *et al.* (1990) *Nature* 348: 176; Donadio *et al.* (1991) *Science* 252: 675; MacNeil *et al.* (1992) *Gene* 115: 119). Structural diversity occurs in this class from variations in the number and type of active sites in the PKSs. This class of PKSs displays a one-to-one correlation between the number and clustering of active sites in the primary sequence of the PKS and the structure of the polyketide backbone. The second class of PKSs, called Type II PKSs, is represented by the synthases for aromatic compounds. Type II PKSs typically have a single set of iteratively used active sites (Bibb *et al.* (1989) *EMBO J.* 8: 2727; Sherman *et al.* (1989(*EMBO J.* 8: 2717; Fernandez-Moreno, *et al.* (1992) *J. Biol. Chem.* 267:19278).

A "nonribosomal peptide synthase" (NRPS) refers to an enzymatic complex of eucaryotic or procaryotic origin, that is responsible for the synthesis of peptides by a nonribosomal mechanism, often known as thiotemplate synthesis (Kleinkauf and von Doehren (1987) *Ann. Rev. Microbiol.,* 41: 259-289). Such peptides, which can be up to 20 or more amino acids in length, can have a linear, cyclic (cyclosporine, tyrocidine, mycobacilline, surfactin and others) or branched cyclic structure (polymyxin, bacitracin and others) and often contain amino acids not present in proteins or modified amino acids through methylation or epimerization.

A "module" refers to a set of distinctive polypeptide domains that encode all the enzyme activities necessary for one cycle of polyketide or peptide chain elongation and associated modifications.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. With respect to nucleic acids and/or polypeptides the term can refer to nucleic acids or polypeptides that are no longer flanked by the sequences typically flanking them in nature.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The term also includes variants on the traditional peptide linkage joining the amino acids making up the polypeptide.

The terms "nucleic acid" or "oligonucleotide" or grammatical equivalents herein refer to at least two nucleotides covalently linked together. A nucleic acid of the present invention is preferably single-stranded or double stranded and will generally contain phosphodiester bonds, although in some cases, as outlined below, nucleic acid analogs are included that may have alternate backbones, comprising, for example, phosphoramide

(Beaucage *et al.* (1993) *Tetrahedron* 49(10):1925) and references therein; Letsinger (1970) *J. Org. Chem.* 35:3800; Sprinzl et al. (1977) *Eur. J. Biochem.* 81: 579; Letsinger *et al.* (1986) *Nucl. Acids Res.* 14: 3487; Sawai *et al.* (1984) *Chem. Lett.* 805, Letsinger *et al.* (1988) *J. Am. Chem. Soc.* 110: 4470; and Pauwels *et al.* (1986) *Chemica Scripta* 26: 1419), phosphorothioate (Mag et al. (1991) *Nucleic Acids Res.* 19:1437; and U.S. Patent No. 5,644,048), phosphorodithioate (Briu *et al.* (1989) *J. Am. Chem. Soc.* 111 :2321, O-methylphophoroamidite linkages (*see* Eckstein, *Oligonucleotides and Analogues: A Practical Approach*, Oxford University Press), and peptide nucleic acid backbones and linkages (*see* Egholm (1992) *J. Am. Chem. Soc.* 114:1895; Meier *et al.* (1992) *Chem. Int. Ed. Engl.* 31: 1008; Nielsen (1993) *Nature*, 365: 566; Carlsson *et al.* (1996) *Nature* 380: 207). Other analog nucleic acids include those with positive backbones (Denpcy *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92: 6097; non-ionic backbones (U.S. Patent Nos. 5,386,023, 5,637,684, 5,602,240, 5,216,141 and 4,469,863; Angew. (1991) *Chem. Intl. Ed. English* 30: 423; Letsinger et al. (1988) *J. Am. Chem. Soc.* 110:4470; Letsinger *et al.* (1994) *Nucleoside & Nucleotide* 13:1597; Chapters 2 and 3, ASC Symposium Series 580, "Carbohydrate Modifications in Antisense Research", Ed. Y.S. Sanghui and P. Dan Cook; Mesmaeker *et al.* (1994), *Bioorganic & Medicinal Chem. Lett.* 4: 395; Jeffs *et al.* (1994) *J. Biomolecular NMR* 34:17; *Tetrahedron Lett.* 37:743 (1996)) and non-ribose backbones, including those described in U.S. Patent Nos. 5,235,033 and 5,034,506, and Chapters 6 and 7, ASC Symposium Series 580, *Carbohydrate Modifications in Antisense Research*, Ed. Y.S. Sanghui and P. Dan Cook. Nucleic acids containing one or more carbocyclic sugars are also included within the definition of nucleic acids (*see* Jenkins *et al.* (1995), *Chem. Soc. Rev.* pp169-176). Several nucleic acid analogs are described in Rawls, C & E News June 2, 1997 page 35. These modifications of the ribose-phosphate backbone may be done to facilitate the addition of additional moieties such as labels, or to increase the stability and half-life of such molecules in physiological environments.

The term "heterologous" as it relates to nucleic acid sequences such as coding sequences and control sequences, denotes sequences that are not normally associated with a region of a recombinant construct, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct is an identifiable segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Similarly, a host cell transformed with a construct which is not normally present in the host cell would be considered heterologous for purposes of this invention.

A "coding sequence" or a sequence which "encodes" a particular polypeptide (*e.g.* a PKS, an NRPS, *etc.*), is a nucleic acid sequence which is ultimately transcribed and/or translated into that polypeptide *in vitro* and/or *in vivo* when placed under the control of appropriate regulatory sequences. In certain embodiments, the boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from procaryotic or eucaryotic mRNA, genomic DNA sequences from procaryotic or eucaryotic DNA, and even synthetic DNA sequences. In preferred embodiments, a transcription termination sequence will usually be located 3' to the coding sequence.

Expression "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence in a host cell. Not all of these control sequences need always be present in a recombinant vector so long as the desired gene is capable of being transcribed and translated.

"Recombination" refers to the reassortment of sections of DNA or RNA sequences between two DNA or RNA molecules. "Homologous recombination" occurs between two DNA molecules which hybridize by virtue of homologous or complementary nucleotide sequences present in each DNA molecule.

The terms "stringent conditions" or "hybridization under stringent conditions" refers to conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent to, or not at all to, other sequences. "Stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and northern hybridizations are sequence dependent, and are different under different environmental parameters. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes part I chapter 2 Overview of principles of hybridization and the strategy of nucleic acid probe assays*, Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.15 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual (2nd ed.)* Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

A "library" or "combinatorial library" of polyketides and/or polypeptides is intended to mean a collection of polyketides and/or polypeptides (or other molecules) catalytically produced by a PKS and/or NRPS and/or hybrid PKS/NRPS (or other possible combination of synthetic elements) gene cluster. The library can be produced by a gene cluster that contains any combination of native, homolog or mutant genes from aromatic, modular or fungal PKSs and/or NRPSs. The combination of genes can be derived from a single PKS and/or NRPS gene cluster, *e.g.*, *act, fren, gra, tcm, whiE, gris, ery*, or the like, and may optionally include genes encoding tailoring enzymes which are capable of catalyzing the further modification of a polypeptide, polyketide, or other molecule. Alternatively, the combination of genes can be rationally or stochastically derived from an assortment of NRPS and/or PKS gene clusters. The library of polyketides and/or polypeptides and/or other molecules thus produced can be tested or screened for biological, pharmacological or other activity.

By "random assortment" is intended any combination and/or order of genes, homologs or mutants which encode for the various PKS and/or NRPS enzymes, modules, active sites or portions thereof derived from aromatic, modular or fungal PKS and/or NRPS gene clusters.

By "genetically engineered host cell" is meant a host cell where the native PKS and/or NRPS gene cluster has been altered or deleted using recombinant DNA techniques or a host cell into which a heterologous PKS and/or NRPS and/or hybrid PKS/NRPS gene cluster has been inserted. Thus, the term would not encompass mutational events occurring in nature. A "host cell" is a cell derived from a procaryotic microorganism or a eucaryotic cell line cultured as a unicellular entity, which can be, or has been, used as a recipient for recombinant vectors bearing the PKS, NRPS, and/or hybrid gene clusters of the invention. The term includes the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired PKS, are included in the definition, and are covered by the above terms.

Expression vectors are defined herein as nucleic acid sequences that are direct the transcription of cloned copies of genes/cDNAs and/or the translation of their mRNAs in an appropriate host. Such vectors can be used to express genes or cDNAs in a variety of hosts such as bacteria, bluegreen algae, plant cells, insect cells and animal cells. Expression vectors include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Specifically designed vectors allow the shuttling of DNA between hosts, such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector preferably contains: an origin of replication for autonomous replication in a host cell, a selectable marker, optionally one or more restriction enzyme sites, optionally one or more constitutive or inducible promoters. In preferred embodiments, an expression vector is a replicable DNA construct in which a DNA sequence encoding a one or more PKS and/or NRPS domains and/or modules is operably linked to suitable control sequences capable of effecting the expression of the products of these synthase and/or synthetases in a suitable host. Control sequences include a transcriptional promoter, an optional operator sequence to control transcription and sequences which control the termination of transcription and translation, and so forth.

A "bleomycin open reading frame", or "bleomycin ORF", or "*BLM* Orf" refers to a nucleic acid open reading frame that encodes a polypeptide or polypeptide domain that has an enzymatic activity used in the biosynthesis of a bleomycin.

A "PKS/NRPS/PKS" system refers to a synthetic system comprising an NRPS flanked by two PKSs. A "NRPS/PKS/NRPS" system refers to a synthetic system comprising a PKS flanked by two NRPSs. A "hybrid PKS/NRPS system" or a "hybrid NRPS/PKS system" refers to a hybrid synthetic system comprising at least one PKS and one NRPS module. The system can comprise multiple modules and the order can vary.

A "biological molecule that is a substrate for a polypeptide encoded by a bleomycin biosynthesis gene" refers to a molecule that is chemically modified by one or more polypeptides enccoded by open reading frame(s) of the *blm* gene cluster. The "substrate" may be a native molecule that typically participates in the biosynthesis of a bleomycin, or can be any other molecule that can be similarly acted upon by the polypeptide.

A "polymorphism" is a variation in the DNA sequence of some members of a species. A polymorphism is thus said to be "allelic," in that, due to the existence of the polymorphism, some members of a species may have the unmutated sequence (*i.e.* the original "allele") whereas other members may have a mutated sequence (*i.e.* the variant or mutant "allele"). In the simplest case, only one mutated sequence may exist, and the polymorphism is said to be diallelic. In the case of diallelic diploid organisms, three genotypes are possible. They can be homozygous for one allele, homozygous for the other allele or heterozygous. In the case of diallelic haploid organisms, they can have one allele or the other, thus only two genotypes are possible. The occurrence of alternative mutations can give rise to trialleleic, *etc.* polymorphisms. An allele may be referred to by the nucleotide(s) that comprise the mutation.

"Single nucleotide polymorphism" or "SNPs are defined by their characteristic attributes. A central attribute of such a polymorphism is that it contains a polymorphic site, "X," most preferably occupied by a single nucleotide, which is the site of the polymorphism's variation (Goelet and Knapp U.S. patent application Ser. No. 08/145,145). Methods of identifying SNPs are well known to those of skill in the art (*see, e.g.,* U.S. Patent 5,952,174).

The following abbreviations are used herein:: A, adenylation; ACP, acyl carrier protein; AT, acyltransferase; BLM, bleomycin; C, condensation; Cy, condensation/cyclization; KR, ketoreductase; KS, ketoacyl synthase; MT, methyltransferase; NRPS, nonribosomal peptide synthetase; orf, open reading frame; Ox, oxidation; PCP, peptidyl carrier protein; PCR, polymerase chain reaction; PKS, polyketide synthase; *Sv*, *Streptomyces verticillus*, ArCP, aryl carrier protein, bp, base pair, CoA, co-enzyme A, DTT, dithiothreitol; FAS, fatty acid synthase; kb, kilobase; PPTase, 4'-phosphopantetheinyl transferase; TCA, trichloroacetic acid; and DEBS, 6-deoxyerythronolide B synthase..

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate the biosynthetic pathway for bleomycin in *Sv* (ATCC 15003). Figure 1A illustrates a biosynthetic pathway for BLM in *Sv* ATCC15003–intermediates except those in brackets were identified. Figure 1B shows a linear model for the Blm megasynthetase-templated assembly of the BLM peptide/polyketide/peptide aglycone from nine amino acids and one acetate–shaded circles represent atypical domains carrying out the proposed novel chemistry, and arrows with broken line indicate where biosynthetic intermediates were derailed. Three-letter amino acid designations were used. [HO], hydroxylation; [H], reduction.

Figure 2 provides a restriction map and gene organization of the *blm* gene cluster from *Sv* ATCC15003 (B, *Bam*HI). Proposed functions for individual open reading frames are summarized in Tables I and II. Modules for individual NRPS and PKS were given along with their proposed substrates in parentheses.

Figures 3A, 3B, 3C, and 3D illustrate the determination of substrate specificity for NRPS-1 and NRPS-6. Figure 3A shows a comparison of the A3 to A6 region of A domains to 84 NRPS modules available at GenBank that activate various amino acids. Figure 3B shows a comparison of amino acid residues that putatively line the substrate binding pockets for A domains (single-letter amino acid designations were used). The number following the protein name indicates the order of a particular A domain in the multimodular NRPS protein. The protein accession numbers are P48663 (HMWP2), P19828 (AngR), AAC06346 (BacA-2), CAB03756 (MbtB), 3510629 (SyrE-7), 3114612 (AcmB-1), CAA67248 (SnbC-1), and 3560507 (FxbC-2). Dhb stands for 2,3-dehydroaminobutyric acid. It is not known if Dhb is the direct substrate for SyrE-7 or resulted from dehydration of an SyrE-7 activated Thr (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863). Figure 3C illustrates purified proteins after overexpression in *E. coli* as analyzed by electrophoresis on a 10% SDS-polyacrylamide gel (the calculated molecular weights for NRPS-1A and NRPS-6A are 64,212 and 61,899, respectively). Figure 3D illustrates substrate specificities as determined by the ATP-PPi exchange reaction with the amino acids of BLM as substrates

(100% relative activity corresponds to 103,000 cpm for NRPS-1A and 256,000 cpm for NRPS-6A).

Figure 4 illustrates a three-module NRPS/PKS/NRPS model for channeling the growing intermediate between NRPS and PKS modules and between PKS and NRPS modules. The KS, ACP, and C domains are shaded to emphasize their unique activities that are responsible for elongating a growing peptide with a short carboxylic acid and a growing polyketide with an amino acid in hybrid peptide/polyketide/peptide biosynthesis.

Figure 5 illustrates the use of *blmVIII* methyltransferase domain to introduce branched methyl groups in a polyketide synthesis. PCK12 has been described by Kao *et al.* (1995) *J. Am. Chem. Soc.,* 7: 9105-9106. DE-1, DE-2 and DE-3 rae three representative products demonstrating the strategy and utility of *blmVIII* in introducing a CH_3 group in polyketide biosynthesis.

Figure 6 illustrates the use of the *blm* NRPS and PKS enzymes to synthesize a variety of hybrid polyketide/peptide molecules including, but not limited to, a family of oxazolines/oxazoles, and thiazoline/thiazoles.

Figure 7 illustrates the use of elements of the *blm* gene cluster to synthesize various sugars.

Figure 8Ashows a restriction map of the *blm* gene cluster from *Sv* ATCC15003 (B, *Bam*HI). 8B shows the relative position of the *blmI*, *blmII*, and *blmXI* genes to the two *blmAB* resistance genes (*blm*^{R}, Blm resistance). Individual open reading frames are represented by open arrows. Figure 8C shows the nucleotide sequence of the *blmI* gene. The potential ribosome-binding site (RBS) and the conserved motif for 4'-phosphopantetheinylation are underlined. The sequence has been deposited into GenBank under accession no. _______.

Figure 9 shows an amino acid sequence comparison of BlmI with PCP domains of known type I NRPSs (Grs-2 [P14688], 36% identity, 58% similarity; Srfa-3 [Q08787], 40% identity, 64% similarity; Vir-s [Y11547], 36% identity, 60% similarity; Saf-b [U24657], 40% identity, 54% similarity). Given in brackets are nucleotide sequence accession numbers. The shaded letters indicate similar amino acids. Consensus residues are amino acids that are similar in more than three sequences. The signature motif for 4'-phosphopantetheinylation is underlined.

Figures 10A and 10B shows the HPLC analysis of BlmI purified from *E. coli* OG7001(pBS2) (Fig. 10A), and *E. coli* OG7001(pBS2/pDPT-Gsp) (Fig. 10B).

Figure 11 shows the enzyme architecture of type I and type II PKS and NRPS. A, adenylation domain; ACP, acyl carrier protein or ACP domain; AT, acyl transferase; C, condensation protein or C domain; KS, β-ketoacyl synthase domain; KSα, β-ketoacyl synthase α subunit; KSβ, β-ketoacyl synthase β subunit; PCP, peptidyl carrier protein or PCP domain.

Figure 12 illustrates the reaction catalyzed by phosphopantetheinyl transferases (PPTases).

Figure 13 shows a restriction map and gene organization of the *pptA* locus from *Sv* ATCC15003

DETAILED DESCRIPTION

Polyketides and polypeptides can be assembled in a remarkably similar manner by repetitive addition of an extending unit to a growing chain by polyketide synthases (PKS) and nonribosomal peptide synthetase (NRPS) respectively. In the case of polyketides, the extending unit is typically a fatty acid (activated as an acyl CoA thioester) while the extending unit for polypeptides is typically an amino acid (activated as an aminonacyl adenylate). Both the PKS and NRPS systems have evolved a modular organization to define the number, sequence, and specificity of the incorporation of the extending unit and utilized the 4'-phosphopanththeine prosthetic group to channel the growing intermediate during the elongation process.

This invention pertains to the discovery that a PKS-bound growing polyketide intermediate could be further elongated by an NRPS module, or conversely, a NRPS-bound growing polypeptide intermediate can be further elongated by a PKS module. This discovery permits the exploitation of NPRS, PKS, and hybrid NRPS/PKS systems to provide a number of novel hybrid peptide/polyketide metabolites from amino acids and short fatty acids.

It was also a discovery of this invention that this hybrid NRPS/PKS/NRPS system is exemplified by the bleomycin (Blm) biosynthesis pathway in *Streptomyces verticillus* (*Sv.*) (ATCC 15003). The bleomycins are a family of glycopeptide-derived antibiotics originally isolated by Umezawa in 1996 from the fermentation broth of *S. verticillus*. Bleomycins (BLMs) exhibit strong anti-tumor activity are currently used in the treatment of lymphoma, particularly Hodgkin's disease, testicular tumors, squamous cell carcinomas of skin, head, cervix, penis, rectum, and for intracavitary therapy of malignant effusions in ovarian and breast cancer. The commercial product, Blenoxane®, contains

BLM A2 and B2 as the principle constituents. Almost uniquely among anticancer drugs, BLM does not cause myelosuppression, promoting its wide application in combination chemotherapy.

In one aspect, this invention provides a cloned and characterized BLM gene cluster consisting of characteristic NRPS and PKS genes from the Blm producer *Streptoveticillum sp.* (ATCC 15003). The cloned and isolated Blm gene cluster provides a method of recombinantly expressing bleomycin and/or bleomycin analogues. Thus, in one embodiment, this invention provides for nucleic acids encoding bleomycin synthetic machinery or subunits thereof, for cells recombinantly modified to express a bleomycin and/or bleomycin analogue, and for a bleomycin or bleomycinh analogue recombinantly expressed in such cells.

Like other polyketide synthase or nonribosomal peptide synthetases, the bleomycin synthetic pathway is organized into modules, each module catalyzing the addition and/or modification of one subunit (*e.g.* fatty acid or amino acid). Each module is organized into a number of domains each domain having a characteristic activity (*e.g.* activation, condensation, condensation/cyclization, *etc.*). The catalytic domains within a module and the modules themselves are often arranged collinearly and the order of biosynthetic modules from NH_2- to COOH-terminus on each PKS and NRPS polypeptide and the number and type of catalytic domains within each determine the order of structural and functional elements in the resulting product. The size and complexity of the ultimately formed product are controlled by the number of repeated acyl chain extension steps that are, in turn, a function of the number and placement of carrier protein domains in these multimodular enzymes. The number composition and order of such domains can be altered either to introduce modifications, *e.g.* into the bleomycin to produce bleomycin analogues, or to produce different or completely new molecules. Such "recombination" is not restricted solely to recombination among the bleomycin catalytic domains and/or modules, but can also involve recombination between beomycin modules and/or subunits and other PKS and/or NRPS modules and/or subunit. Moreover the discovery that synthetic pathways can incorporate both PKS and NRPS modules and/or catalytic domains makes available hybrid PKS/NRPS syntheses.

Thus, in one embodiment this invention contemplates the use of *blm* gene cluster modules and/or catalytic domains to make various peptide and/or polyketide, and/or hybrid polypeptide/polyketide metabolites (including, but not limited to bleomycin intermediates or shunt metabolites), in combinatorial biosynthesis with other polyketide synthases and/or other nonribosomal peptide synthetases.

The *blm* gene cluster contains several glycosylases which can be used alone or in context with other PKS and/or NRPS modules or catalytic domains to make various metabolites with sugars associated with bleomycins (bleomycin sugars).

In addition, the *blm* gene cluster includes a novel methyltransferase domain that can be used to make polyketide metabolites with methyl branch(s).

The *blm* gene cluster also is characterized by the unusual Cy domains as well as the unprecedented Ox domain (*see, e.g.* BlmIV and BlmIII NRPSs), providing an efficient biosynthesis for a bithiazole structure. The *blm* gene cluster, *blm* modules, or *blm* catalytic domains can be used either individually or collectively (alone or in combinations with other nonribosomal peptide synthetases or polyketide synthases) to make thiazolidine, thiazoline and thiazole, bi-thiazolidine, bithiazoline, and bithiazole-containing microbioal metabolites.

Other uses include, but are not limited to the usage of the *blm* gene cluster/modules/catalytic units (either individually or collectively) or the Blm model to make heterocyclic ring-containing microbioal metabolites, such as five member S- and N-containing compounds of the thiazolidine, thiazoline and thiazole family or the O- and N-containing compounds of the oxazolidine, oxazoline, and oxazole family or to make sugars, such L-sugars (with the BlmG epimerase), sugars modified by carbamoyl group (with BlmD), and disaccharides.

This invention also includes the discovery of a novel discrete PCP protein (encoded by the *BlmI* gene). Apo-BlmI can be efficiently modified into holo-BlmI either *in vivo* or *in vitro* by PCP-specific 4'-phosphopantetheine transferases (PPTases) such as Gsp and Sfp. Unlike the PCP domains in type I NRPSs, blmI lacks its cognate A domain and can be aminoacylated by Val-A, an A domain from a completely unrelated type I NRPS. BlmI, therefore, represents the first characterized bype II PCP, providing the genetic and biochemical evidence to support the existence of a bype II NRPS. The latter system is useful, in a manner analogous to the type I NRPS, *i.e.,* modular NRPS, in the combinatorial manipulation of NRPS proteins to generate novel peptides. This invention also includes the discovery and characterizaton of a novel PPTase (encoded by the pptA gene in Figure 13). This PPTase can be used in engineered biosynthesis of polyketides, peptides, hybrid peptide and polyketide metabolites, hybrid polyketide and peptide metabolites, or the combination of both types of metabolites. The PPTase can also be used in converting apo-peptidyl carrier proteins (both type I and type II) and acyl carrier proteins (both type I and type II) into the holo-proteins.

The Examples provided herein and the accompanying primers permit one of ordinary skill in the art to isolate the *blm* gene cluster of this invention, its constituent ORFs, various modules, or enzymatic domains. The isolated nucleic acid components can be used to express one or more polypeptide components for *in vivo* (*e.g. recombinant)* synthesis of one or more polypeptides and/or polyketides as indicated above. It will also be appreciated that the *blm* cluster polypeptides can be used for *ex vivo* assembly of various macromolecules.

I. BLM gene cluster and the PPTase gene.

A) The BLM gene cluster.

The nucleic acids comprising the *blm* gene cluster are identified in Tables I and II and listed in the sequence listing provided herein (SEQ ID NOS: 1 and 2, GenBank Accession numbers AT-149091, AT-210249, AF210311). In particular, Table I identifies genes and functions of open reading frames (ORFs) responsible for the biosynthesis of the hybrid peptide/polyketide/peptide backbone and sugar moieties of bleomycin, while Table II identifies a number of ORFs comprising the *blm* gene cluster, identifies the activity of the catalytic domain encoded by the ORF and provides primers for the amplification and isolation of that orf.

As illustrated in Example 1, the *blm* cluster comprises a PKS module, flanked by several NRPS modules along with several sugar biosynthesis genes and genes encoding other biosynthesis enzymes as well as several resistance and regulatory genes (Table 1).

Table I. Determined functions of ORFs in the bleomycin biosynthesis gene cluster

Gene	Amino acids	Sequence Homolog[1]	Proposed function[2, 3]
orf8	424	YqeR (BAA12461)	Oxidase
blmC	498	RfaE (AA07904.1)	NDP-glucose synthase
blmI	90	GrsB (P14688)	Type II PCP
blmD	545	NodU (Q53515	Carbamoyl transferase
blmE	390	RfaF (AAD16056)	Glycosyl transferase
orf13	187	MbtH (O05821)	Unknown
blmII	462	Nrp (CAA98937)	NRPS condensation enzyme
orf15	339	SyrP (1890776)	Regulation
blmII	935	HMWP2 (P48633), McbC (P23185)	A PCP Ox

blmIV	2626	HMWP2 (P48633)	C A PCP Cy A PCP Cy
orf18	638	AsnB (2293165)	Asparagine synthetase
blmF	494	RfbC (Q50864)/BlmOrf1 (507319)	Glycosyl transferase/β-hydroxylase
blmG	325	YtcB (2293288)	Sugar epimerase
blmV	645	McyB (2708278)	PCP C
blmVI	2675	ACoAS (1658531), PksD (S73014) SnbDE (CAA67249)	A^4 ACP C A PCP C A
blmVII	1218	SyrE (3510629)	C A PCP
blmVIII	1841	HMWP1 (CAA73127)	KS AT MT KR ACP
blmIX	1066	SafB (1171128)	C A PCP
blmX	2140	TycC (2623773)	C A PCP C A PCP
blmXI	688	SyrE (3510629)	NRPS condensation enzyme
orf28	239	SC9C7.04C (CAA22716)	Unknown
orf29	582	YvdB (CAB08068)	Transmembrane transporter
orf30	113	SmtB (P30340)	Regulation
orf31	117	PhnA (P16680)	Unknown

1. Protein accession numbers are given in parentheses. 2. Underlined domains contain motifs that are clearly different from known NRPS or PKS domains. 3. This A domain lacks the typical NRPS A1, A2, A4, A8, and A9 motifs and more closely resembles acyl CoA synthases. *ORF1* to *ORF7* were reported by Schmidt (1994) *Gene* 151:17-21, who assigned ORF2 as *blmA* and ORF4 as *blmB.*

Noteworthy are the genes encoding the NRPS and PKS enzymes. The *blmI, blmII,* and *blmXI* genes encode NRPSs with an unusual architecture. In contrast to all known NRPSs, which are of modular organization with each module consisting minimally of a condensation (C), an adenylation (A), and a peptidyl carrier protein (PCP) domain, *BlmI, BlmII,* and *BlmXI* are discrete proteins homologous to individual domains of type I NRPSs. We have characterized BlmI as a type II PCP (Du and Shen (1999) *Chem. Biol.* 6: 507-517). The *BlmII* and *BlmXI* proteins can serve as candidates for type II condensation enzymes.

The *blmIII, blmIV, blmV, blmVI, blmVII, blmIX,* and *blmX* genes encode modular NRPSs consisting of domains characteristic for known type I NRPSs, such as the A, PCP, C, and condensation/cyclization (Cy) domains, as well as an unprecedented oxidation (Ox) domain. BlmVI is unique among all the Blm NRPSs identified. Its N-terminal module (NRPS-5) consists of an atypical A domain, which bears a close resemblance to a family of acyl CoA synthases (Fitzmaurice and Kolattukudy (1997) *J. Bacteriol.* 179: 2608-2615; Fitzmaurice and Kolattukudy (1998) *J. Biol. Chem.* 273: 8033-8039), and an acyl carrier protein (ACP)-like domain. Its C-terminal module is truncated and presumably interacts with BlmV to constitute the complete NRPS-3 module (Fig. 1B). Also noteworthy are the C domain of NRPS-3 that lacks both His residues of the conserved HHxxxDG (SEQ ID NO: 4) active site for transpeptidation (Stachelhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781) and the extra C domain at the C-terminus of BlmV. These unusual features associated with BlmVI and BlmV may play roles in the formation of the β-aminoalaninamide and the pyrimidine moieties of BLM, which are unprecedented in peptide biosynthesis.

The *blmVIII* gene encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit according to sequence comparison of the AT domain (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) (Fig. 1B).

The identification of an integrated methyltransferase (MT) domain in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain.

Table II. *Blm* gene cluster open reading frames (ORFs) and primers for ORF amplification.

Orf #	Position	Activity	Method	Primers Forward Reverse	Seq ID No.
orf-8	76183-77457	Oxygen-independent coproporphyrinogen III oxidase	Gapped-blast comparison^1	F: ATGAGCCACGCCATCGGA R: TCAGGCGCGTTCGGGGGC	5 6
orf-9	74690-76186	ADP-heptose synthase (*blmC*)	Gapped-blast comparison^1	F: GTGAACACCGACCTGCCC R: TCATGGGGTGTCTCCCTC	7 8
orf-10	74421-74693	Peptidyl carrier protein (*blmI*)	Expression and biochemical characterization.^{2}	F: ATGAGCGCCCCGCGGGGC R: TCACCGGTCCCGCTCCCC	9 10
orf-11	72787-74424	Carbamyltransferase (*blmD*)	Gapped-blast comparison^1	F: ATGAGCGCCGACCCGTCC R: TCATGAGCGGGCCGCCGT	11 12
orf-12	71618-72790	ADP-heptose:LPS heptosyl transferase (*blmE*)	Gapped-blast comparison^1	F: ATGACCACCCCCATGACC R: TCATGGGGTACTCCTGAT	13 14
orf-13	70983-71546	Homolog of mbtH in the synthesis of mycobactin	Gapped-blast comparison^1	F: ATGACCACGACCCCGCGG R: TCAGGTGCCGGACACGCG	15 16
orf-14	69598-70986	Peptide synthetase (condensation, *blmII*)	Gapped-blast comparison^1	F: GTGACCGCCCCCGGCACA R: TCATCGGTGGCTCCTCGT	17 18
orf-15	68582-69601	Regulatory gene (homolog of *syrP*)	Gapped-blast comparison^1	F: GTGAACCGGCACGGCCCC R: TCACGCGCTCACCTCGTC	19 20
orf-16	65778-68585	Mutated peptide synthetase- oxidase (NRPS-0, *blmIII*)	Gapped-blast comparison^1	F: GTGACGAGCGCCCGGCCC R: TCACGGGGCCTCCGTGCG	21 22
orf-17	57901-65781	Peptide synthetase (NRPS-2-1,*blmIV*)	Expression and biochemical characterization.^{2}	F: ATGCTGCACGGCGCCGCG R: TCACTCCGGTCCACCTCC	23 24

orf-18	55899-57815	Asparagine synthetase	Gapped-blast comparison[1]	F: GTGAGGCCCGTGTGCGGC R: TCAGCCACCGTTGCCGCC	25 26
orf-19	54418-55902	Homolog of hydroxylase-dehydrogenase (*blmF*)	Gapped-blast comparison[1]	F: GTGAAGGACCTCGGCCGG R: TCACTCCCCCGGTGCCGG	27 28
orf-20	53427-54404	Nucleotide-sugar epimerase (*blmG*)	Gapped-blast comparison[1]	F: GTGACATGGACCGTGGTG R: TCAGGCATCGGCCCTCCC	29 30
orf-21	51493-53430	Peptide synthetase (NRPS-3CT, *blmV*)	Gapped-blast comparison[1]	F: ATGCGCGGGCATGACGAC R: TCACGGTGTCTCTCCCTC	31 32
orf-22	43263-51290	Peptide synthetase (NRPS-5-4-3, *blmVI*)	Expression and biochemical characterization.[2]	F: ATGAGCCGGCCGGCCGGC R: TCATGCTCGGTCATCGCC	33 34
orf-23	39610-43266	Peptide synthetase (NRPS-6, *blmVII*)	Expression and biochemical characterization.[2]	F: GTGACCACGCCCCGCATC R: TCATTCGGGACGCGGGCA	35 36
orf-24	34088-39613	Polyketide synthase (*blmVIII*)	Gapped-blast comparison[1]	F: ATGAGCCATGCCGACGCG R: TCACAGCACCACCTCTTC	37 38
orf-25	30891-34091	Peptide synthetase (NRPS-7, *blmIX*)	Gapped-blast comparison[1]	F: ATGACCCCGGCCGCCGAC R: TCATCGTCCGCCGCCTTT	39 40
orf-26	24406-30894	Peptide synthetase (NRPS-9-8, *blmX*)	Gapped-blast comparison[1]	F: ATGCCTCGGTGTGCCCGA R: TCATTCGGCGGCACCTCC	41 42
orf-27	22127-24193	Peptide synthetase (condensation, *blmXI*)	Gapped-blast comparison[1]	F: GTGGGTTTCCGTCGAGCG R: TTACACCCTCCGTTTCTC	43 44
orf-28	21367-22086	Phosphatidylserine decarboxylase	Gapped-blast comparison[1]	F: ATGGCACAGGACCTGAAC R: TCAACGCCACCGGATCTT	45 46
orf-29	19161-20909	Transmembrane transporter	Gapped-blast comparison[1]	F: GTGAGCTCCCTCGCCGTC R: TCATCGTCGGGCACTCGG	47 48
orf-30	18823-19164	Metal dependent regulatory element	Gapped-blast comparison[1]	F: GTGCCGGTTCCGCTGTAT R: TCACCGGGCACTGACCTC	49 50
orf-31	18660-18307	PHNA homolog	Gapped-blast comparison[1]	F: GTGACCGAGAACCTTCCG R: TCAGACCTTCTTGACCAC	51 52
orf-32	17736-9211	Peptide synthetase (NRPS-11-10)	Gapped-blast comparison[1]	F: ATGGCCTCAGACGCTTTG R: TCATTGAGACTCCTCCTC	53 54
orf-33	9214-7859	Putative transporter	Gapped-blast comparison[1]	F: ATGATGAAGTCAAGCCGC R: TCAGTGGCTTACAAGGAG	55 56
orf-34	7797-6784	Homolog of clavaminic acid synthase	Gapped-blast comparison[1]	F: ATGACTGACCTGCCGTTG R: TCACACCAGCAGCGAGGT	57 58
orf-35	6773-6021	Thioesterase	Gapped-blast comparison[1]	F: ATGGATTTCCCCCTCACC R: TCATGCCCCTACCTCGGC	59 60
orf-36	6024-4741	Putative transporter	Gapped-blast comparison[1]	F: ATGACCGCGCGCGTCGAC R: TCACTCCTCGGCTTCGGC	61 62
orf-37	4733-3915	Unknown	Gapped-blast comparison[1]	F: GTGTCCAAGAACGCGGCG R: TCATCGGCTCGCCTCGTG	63 64
orf-38	3918-2182	Peptide synthetase (NRPS-12)	Gapped-blast comparison[1]	F: ATGACCCTCACCCTGCGG R: TCACTCGGGCACTCCTTC	65 66
orf-39	2185-1199	Regulatory gene (homolog of *SyrP*	Gapped-blast comparison[1]	F: GTGACCGGTTCCGTAACG R: TCATGAGTCCGCCGAGGT	67 68
orf-	1015-1	Peptide synthetase	Gapped-blast	F: ATGACAGAGGTCCGAGGT	69

40			comparison[1]	R: CCCGGCAACCGCCCTCCC	70
orf-41	On a separate sequence	4'-phosphopantetheinyl transferase (*pptA*)	Expression and biochemical characterization.[2]	F: GTGATCGCCGCCCTCCTG R: TTACGGGACGGCGGTCCG	71 72

The Blm megasynthetase comprises nine NRPS modules and one PKS module forming a hybrid NRPS/PKS/NRPS metasynthetase (Fig. 1A). Inspection of the blm gene cluster (Fig. 2) showed that the Blm NRPS and PKS modules apparently are not organized according to the "colinearity rule" for BLM biosynthesis (Fig. 1). Detailed functional organization of the megasynthetase and the BLM synthetic pathway is provided in Example I.

B) PPTase

This invention also provides the gene (pptA, Fig. 13) encoding phosphopantetheine transferase (PPTase) (GenBank Accession No: AF210311) (*see,* SEQ ID NO: 3). PPTase converts carrier proteins for the growing acyl chain from inactive apo-forms to functional holo-forms by the covalent attachment of the 4'-phosphopantetheine moiety of coenzyme A to a conserved serine residue of the carrier-protein substrate (*see, e.g.,* Fig. 1A).

Using the sequence information provided herein (*e.g.* primer sequences and PPTase sequence) the PPTase nucleic acids can be routinely isolated according to standard methods (*e.g.* PCR amplification). Detailed protocols for the isolation of the PPTase are provided in Example 3.

Other PPTases can be identified using the probes and primers illustrated in Example 3. Briefly, using a primer to the THC motif (5'-C GGC ATG GTC GGC TCC HTN CAN CAY TG -3', SEQ ID NO: 73, where H= C+A, N = A + C + T + G, Y = C + T, K = G + T, R = A + G, W = T + A), and a primer designed around the typical C-terminal PPTase motif (*e.g.,* KEA-1: 5'-T GCA GCA GAA CAG GAG GCK NYC CCA NKG - 3', SEQ ID NO: 74, and KEA-2: 5'- TG GGT CAG CGG GTA CCA NRC YTT RWA - 3', SEQ ID NO: 75), and using *S. verticillus* chromosomal DNA as template, the set of primers THC/KEA-2 a probe can be amplified (about 250 bp), that specifically binds to a PPTase. Libraries of organisms comprising NRPS, PKS, and/or hybrid PKS/NRPS pathways can be probed for the presence of a PPTase sequence. Once hybridizing clones are identified, the PPTase sequence can be isolated according to standard methods well know to those of skill in the art (*see, e.g.,* Example 3).

C) Isolation/preparation of nucleic acids.

In one embodiment, this invention provides nucleic acids for the recombinant expression of a bleomycin. Such nucleic acids include isolated gene cluster(s) comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

In other embodiments of this invention, modified bleomycins (*e.g.* bleomycin analogs), novel polyketides, polypeptides, and combinations thereof (polyketide/polypeptide hybrids) are created by modifying PKSs and/or NRPSs so as to introduce variations into known polymers synthesized by the enzymes. Such variations may be introduced by design, for example to modify a known molecule in a specific way, *e.g.* by replacing a single monomeric unit within a polymer with another, thereby creating a derivative molecule of predicted structure. Alternatively, variations can be made randomly, for example by making a library of molecular variants of a known polymer by systematically or haphazardly replacing one or more modules or enzymatic domains in a known PKS or NRPS with a collection of alternative modules or domains. Production of alternative/modified PKSs, NRPSs and hybrid systems is described below.

Using the primer and sequence information provided herein, one of ordinary skill in the art can routinely isolate/clone the PKS and/or NRPS modules and/or enzymatic domains described herein. For example, the PCR primers provided in Table II, above, can be used to amplify any of the orfs identified therein. Moreover, using the sequence information for the *blm* gene cluster provided herein, the design of other primers suitable of the amplification of individual ORFs, combinations of ORFs, genes, *etc.* is routine.

Typically such amplifications will utilize the DNA of an organism containing the requisite genes (*e.g. Streptomyces verticillus*) as a template. Typical amplification conditions include a PCR mixture consisting of 5 ng of *S verticillus* genomic or plasmid DNA as template, 25 pmoles of ech primers, 25 μM dNTP, 5% DMSO, 2 units of *Taq* polymerase, 1 x buffer, with or without 20% glycerol in a final volume of 50 μL. PCR is carried out (*e.g.* on a Gene Amp PCR System 2400 (Perkin-Elmer/ABI)) with a cycling scheme as follows: initial denaturing at 94°C for 5 min, 24-36 cycles of 45 sec at 94°C, 1 min at 60°C, 2 min at 72°C, followed by additional 7 min at 72°C. One of skill will appreciate that optimization of such a protocol, *e.g.* to improve yield, *etc.* is routine (*see, e.g.,* U.S. Patent No. 4,683,202; Innis (1990) *PCR Protocols A Guide to Methods and Applications* Academic Press Inc. San Diego, CA, *etc*). In addition, primer may be designed to introduce restriction sites and so facilitate cloning of the amplified sequence into a vector.

Using the information provided herein other approaches to cloning the desired sequences will be apparent to those of skill in the art. For example, the PKS or NRPS modules or enzymatic domains of interest can be obtained from an organism that expresses the same, using recombinant methods, such as by screening cDNA or genomic libraries, derived from cells expressing the gene, or by deriving the gene from a vector known to include the same. The gene can then be isolated and combined with other desired NRPS and/or PKS modules or domains, using standard techniques. If the gene in question is already present in a suitable expression vector, it can be combined *in situ,* with, e.g., other PKS subunits, as desired. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence can be assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence (*see, e.g.,* Edge (1981) *Nature* 292:756; Nambair *et al.* (1984) *Science* 223: 1299; Jay *et al.* (1984) *J. Biol. Chem.* 259:6311). In addition, it is noted that custom gene synthesis is commercially available (*see, e.g.* Operon Technologies, Alameda, CA).

Examples of such techniques and instructions sufficient to direct persons of skill through many cloning exercises are found in Berger and Kimmel (1989) *Guide to Molecular Cloning Techniques, Methods in Enzymology 152* Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al.* (1989) *Molecular Cloning - A Laboratory Manual* (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Ausubel (19 1994) *Current Protocols in Molecular Biology,* Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., U.S. Patent 5,017,478; and European Patent No. 0,246,864.

II. Expression of blm gene clusters, modules, and enzymatic domains.

As indicated above, in one embodiment this invention provides novel NRPS and PKS genes for the efficient recombinant production of both novel and known polyketides, peptides, and polyketide/polypeptide hybrids by expressing them *in vivo.* In other embodiments, such syntheses are carried out *in vitro.* Even *in vitro* syntheses, however, typically utilize recombinantly expressed PKSs, NRPSs, or enzymatic domains thereof. Thus, it is frequently desirable to express protein components of the PKSs or NRPs described above.

Typically expression of the protein components of the pathway and/or of the products of the NRPS/PKS pathway is accomplished by placing the subject PKS or NRPS nucleic acid(s) in an expression vector, and transfecting a cell with the vector such that the cell expresses the desired product(s).

A) Expression vectors

The choice of vector depends on the sequence(s) that are to be expressed. Any transducible cloning vector can be used as a cloning vector for the nucleic acid constructs of this invention. However, where large clusters are to be expressed, it phagemids, cosmids, P1s, YACs, BACs, PACs, HACs or similar cloning vectors be used for cloning the nucleotide sequences into the host cell. Phagemids, cosmids, and BACs, for example, are advantageous vectors due to the ability to insert and stably propagate therein larger fragments of DNA than in M13 phage and lambda phage, respectively. Phagemids which will find use in this method generally include hybrids between plasmids and filamentous phage cloning vehicles. Cosmids which will find use in this method generally include lambda phage-based vectors into which cos sites have been inserted. Recipient pool cloning vectors can be any suitable plasmid. The cloning vectors into which pools of mutants are inserted may be identical or may be constructed to harbor and express different genetic markers (see, *e.g.*, Sambrook *et al.*, *supra*). The utility of employing such vectors having different marker genes may be exploited to facilitate a determination of successful transduction.

In preferred embodiments of this invention, vectors are used to introduce PKS, NRPS, or NRPS/PKS genes or gene clusters into host (*e.g. Streptomyces*) cells. Numerous vectors for use in particular host cells are well known to those of skill in the art. For example described in Malpartida and Hopwook, (1984) *Nature*, 309:462-464; Kao *et al.*, (1994), *Science*, 265: 509-512; and Hopwood *et al.*, (1987) *Methods Enzymol.*, 153:116-166 all describe vectors for use in various *Streptomyces* hosts.

In a preferred embodiment, *Streptomyces* vectors are used that include sequences that allow their introduction and maintenance in *E. coli*. Such *Streptomyces/E. coli* shuttle vectors have been described (*see*, for example, Vara *et al.,* (1989) *J. Bacteriol.*, 171:5872-5881; Guilfoile & Hutchinson (1991) *Proc. Natl. Acad. Sci. USA,* 88: 8553-8557.)

The gene sequences, or fragments thereof, which collectively encode a PKS and/or NRPS and/or PKS/NRPS gene cluster, one or more ORFs, one or more modules, or one or more enzymatic domains of this invention, can be inserted into one or more expression vectors, using methods known to those of skill in the art. Expression vectors will include control sequences operably linked to the desired NRPS and/or PKS coding sequence or fragment thereof. Suitable expression systems for use with the present invention include systems that function in eucaryotic and prokaryotic host cells. However, as explained above, prokaryotic systems are preferred, and in particular, systems compatible with *Streptomyces spp.* are of particular interest. Control elements for use in such systems include promoters, optionally containing operator sequences, and ribosome binding sites. Particularly useful promoters include control sequences derived from PKS and/or NRPS gene clusters, such as one or more *act* promoters. However, other bacterial promoters, such as those derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) and maltose, will also find use in the present constructs. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*), the beta -lactamase (*bla*) promoter system, bacteriophage lambda PL, and T5. In addition, synthetic promoters, such as the tac promoter (U.S. Patent 4,551,433), which do not occur in nature also function in bacterial host cells. In *Streptomyces*, numerous promoters have been described including constitutive promoters, such as *ermE* and *tcmG* (Shen and Hutchinson, (1994) *J. Biol. Chem.* 269: 30726-30733), as well as controllable promoters such as *actI* and *actIII* (Pleper *et al.,* (1995) *Nature*, 378: 263-266; Pieper *et al.*, (1995) *J. Am. Chem. Soc.*, 117: 11373-11374; and Wiesmann *et al.,* (1995) *Chem. & Biol.* 2: 583-589).

Other regulatory sequences may also be desirable which allow for regulation of expression of the PKS replacement sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

Selectable markers can also be included in the recombinant expression vectors. A variety of markers are known which are useful in selecting for transformed cell lines and generally comprise a gene whose expression confers a selectable phenotype on transformed cells when the cells are grown in an appropriate selective medium. Such markers include, for example, genes that confer antibiotic resistance or sensitivity to the plasmid. Alternatively, several polyketides are naturally colored and this characteristic provides a built-in marker for selecting cells successfully transformed by the present constructs.

The various PKS and/or NRPS clusters or subunits of interest can be cloned into one or more recombinant vectors as individual cassettes, with separate control elements, or under the control of, *e.g.,* a single promoter. The PKS and/or NRPS subunits can include flanking restriction sites to allow for the easy deletion and insertion of other PKS subunits so that hybrid PKSs can be generated. The design of such unique restriction sites is known to those of skill in the art and can be accomplished using the techniques described above, such as site-directed mutagenesis and PCR.

Methods of cloning and expressing large nucleic acids such as gene clusters, including PKS- or NRPS-encoding gene clusters, in cells including *Streptomyces* are well known to those of skill in the art (*see, e.g.,* Stutzman-Engwall and Hutchinson (1989) *Proc. Natl. Acad. Sci. USA,* 86: 3135-3139; Motamedi and Hutchinson (1987) *Proc. Natl. Acad. Sci. USA,* 84: 4445-4449; Grim *et al.* (1994) *Gene,* 151: 1-10; Kao *et al.* (1994) *Science,* 265: 509-512; and Hopwood *et al.* (1987) *Meth. Enzymol.,* 153: 116-166). In some examples, nucleic acid sequences of well over 100kb have been introduced into cells, including prokaryotic cells, using vector-based methods (*see*, for example, Osoegawa *et al.,* (1998) *Genomics*, 52: 1-8; Woon *et al.,* (1998) *Genomics*, 50: 306-316; Huang *et al.,* (1996) *Nucl. Acids Res.*, 24: 4202-4209). In addition, the cloning and overexpression of NRPS-1 and NRPS-6 is illustrated in Example 1.

In certain embodiments this invention may make use of genetically engineered cells that either lack PKS and/or NRPS genes or have their naturally occurring PKS and/or NRPS genes substantially deleted. These host cells can be transformed with recombinant vectors, encoding a variety of PKS and/or NRPS gene clusters, for the production of active polyketides. The invention provides for the production of significant quantities of product, *e.g.* a bleomycin, at an appropriate stage of the growth cycle. The BLMs or other hybrid polyketide/peptide metabolites so produced can be used as therapeutic agents, to treat a number of disorders, depending on the type of metabolites in question. For example, several of the polyketides and peptides produced by the present method will find use as immunosuppressants, as anti-tumor agents, as well as for the treatment of viral, bacterial and parasitic infections. The ability to recombinantly produce polyketides and peptides also provides a powerful tool for characterizing PKSs and/or NRPSs and the mechanism of their actions.

B) Host cells.

The vectors described above can be used to express various protein components of the polyketide and/or polypeptide synthetic modules for subsequent isolation and/or to provide a biological synthesis of one or more desired biomolecules (*e.g* polyketides, peptides, *etc.*). Where one or more proteins of the *blm* cluster are expressed (*e.g.* overexpressed) for subsequent isolation and/or characterization, the proteins are expressed in any prokaryotic or eukaryotic cell suitable for protein expression. In one preferred embodiment, the proteins are expressed in *E. coli.* Overexpression of *blmI* in *E. coli* is described in Example 2.

Host cells for the recombinant production of the subject polyketides can be derived from any organism with the capability of harboring a recombinant PKS, NRPS or PKS/NRPS gene cluster. Thus, the host cells of the present invention can be derived from either prokaryotic or eucaryotic organisms. However, preferred host cells are those constructed from the actinomycetes, a class of mycelial bacteria which are abundant producers of a number of polyketides and peptides. A particularly preferred genus for use with the present system is Streptomyces. Thus, for example, *S. verticillus S. ambofaciens, S. avermitilis, S. azureus, S. cinnamonensis, S. coelicolor, S. curacoi, S. erythraeus, S. fradiae, S. galilaeus, S. glaucescens, S. hygroscopicus, S. lividans, S. parvulus, S. peucetius, S. rimosus, S. roseofulvus, S. thermotolerans, S. violaceoruber*, among others, will provide convenient host cells for the subject invention, with *S. coelicolor* being preferred (*see, e.g., Hopwood, D. A. and Sherman, D. H. Ann. Rev. Genet. (1990) 24:37-66; O'Hagan, D. The Polyketide Metabolites (Ellis Horwood Limited, 1991), for a description of various polyketide-producing organisms and their natural products.)*

In a preferred embodiment, the above-described cells are genetically engineered by deleting one or more naturally occurring PKS and/or NRPS genes therefrom, using standard techniques, such as by homologous recombination. (*see, e.g.*, Khosla, *et al.* (1992) *Molec. Microbiol.* 6: 3237).

In certain embodiments, a eukaryotic host cell is preferred (*e.g.* where certain glycosylation patterns are desired). Suitable eukaryotic host cells are well known to those of skill in the art. Such eukaryotic cells include, but are not limited to yeast cells, insect cells, plant cells, fungal cells, and various mammalian cells (*e.g.* COS, CHO HeLa cells lines and various myeloma cell lines)

C) Protein/polyketide recovery.

Polypeptide and/or polyketide recovery is accomplished according to standard methods well known to those of skill in the art. Thus, for example where *blm* cluster proteins are to be expressed and isolated, the proteins can be expressed with a convenient tag to facilitate isolation (*e.g.* a His_6) tag.· Other standard protein purification techniques are suitable and well known to those of skill in the art (*see, e.g.,* Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321, *etc.*).

Similarly where components (*e.g.* modules and/or enzymatic domains) of the *blm* cluster are used to express various biomolecules (*e.g.* polyketides, sugars, polypeptides, *etc.*) the desired product and/or shunt metabolite(s) are isolated according to standard methods well know to those of skill in the art (*see, e.g.,* Carreras and Khosla (1998) *supra.*) Purification and in vitro reconstitution of the essential protein components of an aromatic polyketide synthase. *Biochemistry* 37: 2084-2088, Deutscher (1990) *Methods in Enzymology Volume 182: Guide to Protein Purification*, M. Deutscher, ed. .

III. Synthesis of recombinant bleomycins.

In one embodiment this invention provides methods of synthesizing bleomycins and recombinantly synthesized bleomycins. As indicated above, this is generally accomplished by providing an organism (*e.g.* a bacterial cell) containing sufficient compoents of the *blm* gene cluster to direct synthesis of a complete bleomycin.

In one embodiment, the entire *blm* cluster is cloned into a *Streptomyces* strain (*e.g., S. lividans* or *S. coelicolor*). Kao *et al.*(1994) *Science,* 265: 509-512, have cloned the 30 kb DEBS genes from *Sacc. erythmea* into *S. coelicolor* and produced 6-deoxyerythronolide B in *S. coelicolor* and these methods can be used construct an expression plasmid for heterologous expression of the *blm* cluster. This method involves the transfer of DNA between a temperature-sensitive plasmid and a shuttle vector by means of a homologous double recombination event in *E. coli* (*Id.*). In a preferred embodiment, the two ends spanning the *blm* cluster are cloned into a temperature-sensitive plasmid that is chloramphenicol resistant (CM^R) such as pCK6. *S. verticillus* DNA is then rescued from a donor into the temperature-sensitive recipient by co-transforming *E. coli* with the Cm^R recipient plasmid and the apramycin resistant (Ap^R) pKC505 donor cosmid that contains the *blm* gene cluster, followed by chloramphenicol and apramycin selection at 30°C. Colonies harboring both plasmids (Cm^R, Ap^R) will be shifted to 44°C on chloramphenicol and apramycin plates and only those cointegrates formed by a single recombination event between the two plasmids are viable. Surviving colonies are then propagated at 30°C on Cm^R plates to select for recombinant plasmids formed by the resolution of cointegrates through a second recombinant event. The desired *blm* cluster is cloned into the Cm^R temperature-sensitive plasmid and is ready to be moved into any expression plasmid by a similar means of homologous recombinant event.

For example, if pWHM861 is the choice of shuttle plasmid for the expression of the *blm* cluster in *S. lividans* (Meurer and Hutchinson (1995) *J. Bacteriol.,* 177: 477-481), the two ends spanning the *blm* cluster downstream of the *ErmE** promoter in the ampicillin resistant (AM^R) plasmid pWHM861 are cloned. The resulting plasmid is co-transformed with the temperature-sensitive plasmid containing the *blm* cluster described above into *E. coli* under the selection of chloramphenicol and ampicillin at 30°C. These Cm^R and AM^R colonies are shifted to 44°C on chloramphenicol and ampicillin plates to undergo a single recombination event and the surviving colonies are resolved on ampicillin plates at 30°C by completing the double recombination process. The resulting plasmid is suitable for transformation into *S. lividans* by selection of thiostrepton, in which the expression of the desired *blm* cluster is under the control of the *ErmE** promoter. The *S. lividans* transformants are cultured and any metabolites produced are isolated and characterized.

Once production of BLM in *S. lividans* is established, mutated alleles of the *blm* synthetase can be introduced into the *blm* cluster for the production of BLM analogs.

IV. Altered endogenous expression of bleomycins.

Using the Blm gene cluster information provided herein, one of skill in the art may regulating the synthesis of endogenous bleomycin. The expression of various ORFs comprising the *blm* gene cluster may be increased or decreased to alter bleomycin synthesis levels.

Methods of altering the expression of endogenous genes are well known to those of skill in the art. Typically such methods involve altering or replacing all or a portion of the regulatory sequences controlling expression of the particular gene that is to be regulated. In a preferred embodiment, the regulatory sequences (*e.g.,* the native promoter) upstream of one or more of the *blm* ORFs are altered.

This is typically accomplished by the use of homologous recombination to introduce a heterologous nucleic acid into the native regulatory sequences. To downregulate expression of one or more *blm* ORFs, simple mutations that either alter the reading frame or disrupt the promoter are suitable. To upregulate expression of the *blm* ORF(s) the native promoter(s) can be substituted with heterologous promoter(s) that induce higher than normal levels of transcription.

In a particularly preferred embodiment, nucleic acid sequences comprising the structural gene in question or upstream sequences are utilized for targeting heterologous recombination constructs.

The use of homologous recombination to alter expression of endogenous genes is described in detail in U.S. Patent 5,272,071, WO 91/09955, WO 93/09222, WO 96/29411, WO 95/31560, and WO 91/12650.

V. Synthesis of BLM analogs.

In one one embodiment, this invention provides methods of synthesizing modified bleomycins or bleomycin analogs. In preferred embodiments, the BLM analogs are synthesized either by introducing specific perturbations into individual NRPS and/or PKS enzymatic domains or modules, or by reprogramming the linear order in which the NRPS or PKS enzymatic domains and/or modules appear in the *blm* synthetase genes. The former will lead to BLM analogs with targeted modifications at the BLM backbone and the latter will allow incorporation of other extension units in variable sequence into the biosynthesis of BLM. In particularly preferred embodiments, the genetically modified *blm* synthetases are produced in *S. verticilus*, however, it will be recognized that the entire *blm* gene cluster can be cloned into other hosts, *e.g.* into *S. lividans* or *S. coelicolor.*

In preferred embodiments modification of the *blm* gene cluster to yield BLM analogues is accomplished by one of two different approaches. In one approach, the BLM enzymatic domains and/or modules modules are altered in a directed manner (*i.e.* they are changed in a preselected way), while in another approach, random/haphazard alterations are introduced into the *blm* cluster and the resulting products are screened to identify those with desired properties.

A) Synthesis of BLM analogs by specific engineering of the *blm* synthetase genes.

The *blm* synthetase genes can be re-engineered by means of specific mutations or by reprogramming the linear order of the NRPS or PKS enzymatic domains or modules. In this approach, a wild-type *blm* synthetase allele is replaced with these mutants in and expressed in an appropriate host (*e.g., S. verticillus* or in a heterologous host). Since both NRPSs (Stachelhaus *et al.* (1995) *Science*, 269: 69-72) and PKSs (Donadio *et al.* (1993)

Proc. Natl. Acad. Sci. USA, 90: 7119-7123, Donadio *et al.* (1995) *J. Am., Chem. Soc.,* 117: 9105-9106, Cortes *et al.* (1995) *Science,* 268: 1487-1489) have shown considerable tolerance to reprogramming, it is expected that these modifications of the BLM synthetase will result in the production of BLM analogs with predicted structural alterations. For example, targeted modification at the (2S,3S,4R)-4-amino-3-hydroxy-2-methyl/pentanoic acid AHM moiety of BLM can be accomplished by introduction of mutations into the *BLMVIII* PKS module of the BLM synthetase locus. Inactivation of the MT or KR motif by in-frame deletion or site-directed mutagenesis will result in the production of BLM analogs containing a demethyl-AHM, oxo-AHM, or oxo-demethyl-AHM moiety, *etc.*

Alternatively, individual functional NRPS domains and/or the PKS module can be deleted or the PKS module can be duplicated in-frame to produce BLM analogs with shorter or longer backbone, respectively. Alternatively, or in addition, the NRPS domains or the PKS module can be rearranged for the production of BLM analogs with a completely different backbone. The NRPS and PKS features can be combined into one integrated system, providing access to a structural variation not available by either the NRPS or PKS system alone.

To create such mutations, plasmids are constructed carrying in-frame deletions of DNA segments encompassing a portion of the *blm* synthetase activities. Construction of specific deletions is preferably accomplished by one of the following two strategies. The first involves subcloning of a DNA fragment in a gene replacement vector, selection of two restriction sites suitably located at the two ends of the DNA segments, and deletion of this segment from within the plasmid by rejoining the two resulting ends. An in-frame deletion can be obtained by a suitable combination of Klenow filling and S1 treatment of both ends prior to ligation.

The second approach involves polymerase chain reaction (PCR) amplification of two DNA segments that separate the region to be deleted followed by joining of the two fragments in the correct orientation in a gene replacement vector. This can be accomplished by designing PCR primers with suitable restriction sites. The restriction site used to generate the deletion and the sequences to serve as templates for the PCR amplification are chosen so as to generate two segments of *blm* synthetase DNA of approximately equal length in the construction in order to maximize the chance of gene replacement. The gene replacement vector containing the allelic or deletion mutation is introduced into a *Streptomyces* strain (*e.g., S. verticillus*). Integration of the plasmid into the *S. verticillus* chromosome via a single reciprocal homologous recombination will yield a recombinant that will be isolated by selection for the vector marker. The resulting integrants are then grown under non-selective conditions and further resolution by selection for the loss of the vector marker via the second homologous recombination event will produce the desired deletion mutants.

Southern analysis of the isolated deletion mutants with the target DNA is performed to ensure that the expected double crossover recombination event has taken place. The first approach is convenient if there are suitably spaced restriction sites in the DNA sequence. The second approach enables the deletion of any DNA segment but may be limited by the size of the DNA segments that can be amplified by PCR. These *S. verticillus* recombinants are cultured under typical conditions for BLM production and the fermentation broth is screened for the production of any novel BLM analogs resulted from the specific mutations in the *blm* synthetase locus.

B) Synthesis of BLM analogs by "random" modification of *blm* synthetase genes.

Bleomycin analogs can also be synthesized by randomly/haphazardly altering genes in the BLM cluster expressing the products of the randomly modified megasynthetase and then screening the products for the desired activity. Methods of "randomly" altering *blm* cluster genes are described below.

VI. Generation of other synthetic systems.

In addition to the production of bleomycin or modified bleomycins, the *blm* gene cluster or elements thereof can be used by themselves or in combination with NRPS and/or PKS modules and/or enzymatic domains of other PKS and/or NRPS systems to produce a wide variety of compounds including, but not limited to various polyketides, polypeptides, polyketide/polypeptide hybrids, various oxazoles and thiazoles, various sugars, various methylated polypeptides/polyketides, and the like. As with the production of modified bleomycins described above, such compounds can be produced, *in vivo* or *in vitro*, by catalytic biosynthesis using large, modular PKSs, NRPSs, and hybrid PKS/NRPS systems. The megasynthetases directing such syntheses can be rationally designed *e.g.* by predetermined alteration/modification of polyketide and/or polypeptide and/or hybrid PKS/NRPS pathways. Alternatively, large combinatorial libraries of cells harboring various megasynthetases can be produced by the random modification of particular pathways and then selected for the production of a molecule or molecules of interest. It will be appreciated that, in certain embodiments, such libraries of megasynthetases/modified pathways, can be used to generate large, complex combinatorial libraries of compounds which themselves can be screened for a desired activity.

A) Directed modification of biomolecules.

Elements (*e.g.* open reading frames) of the *blm* biosynthetic gene cluster and/or variants thereof can be used in a wide variety of "directed" biosynthetic processes (*i.e.* where the process is designed to modify and/or synthesize one or more particular preselected metabolite(s)). Polypepitdes encoded by particular open reading frames or combinations of open reading frames can be utilized to perform particular chemical modifications of biological molecules.

Thus, for example, open reading frames encoding a polypeptide synetase can be used to chemically modify an amino acid by coupling it to another amino acid. In another example, the methyl transferase in *BlmVIII* can be utilized to introduce methyl groups into polyketides, and other, substrates. The glycosyl transferases can be used to glycosylate appropriate substrates, and so forth. These examples, are merely illustrative. One of skill in the art, utilizing the information provided here, can perform literally countless chemical modifications and/or syntheses using either "native" bleomycin biosynthesis metabolites as the substrate molecule, or other molecules capable of acting as substrates for the particular enzymes in question. Other substrates can be identified by routine screening. Methods of screening enzymes for specific activity against particular substrates are well known to those of skill in the art.

The biosyntheses can be performed *in vivo*, *e.g.* by providing a host cell comprising the desired *blm* gene cluster open reading frame(s) and/or *in vivo*, *e.g.*, by providing the polypeptides encoded by the *blm* gene cluster ORFs and the appropriate substrates and/or cofactors.

B) Directed engineering of novel synthetic pathways.

In numerous embodiments of this invention, novel polyketides, polypeptides, and combinations thereof are created by modifying known PKSs or NRPSs so as to introduce variations into known polymers synthesized by the enzymes. Such variations may be introduced by design, for example to modify a known molecule in a specific way, *e.g.* by replacing a single monomeric unit within a polymer with another, thereby creating a derivative molecule of predicted structure. Such variations can also be made by adding one or more modules to a known PKS or NRPS, or by removing one or more module from a known PKS or NRPS. Such novel PKSs or NRPSs can readily be made using a variety of techniques, including recombinant methods and *in vitro* synthetic methods.

Using any of these methods, it is possible to introduce PKS domains into a NRPS, or vice versa, thereby creating novel molecules including both peptide and polyketide structural domains. For example, a PKS enzyme producing a known polyketide can be modified so as to include an additional module that adds a peptide moiety into the polyketide. Novel molecules synthesized using these methods can be screened, using standard methods, for any activity of interest, such as antibiotic activity, effects on the cell cycle, effects on the cytoskeleton, etc.

Novel polyketides, polypeptides, or combinations thereof can also be made by creating novel PKSs or NRPSs *de novo*, using recombinant or *in vitro* synthetic methods. Such novel arrangements of domains can be designed, *i.e.* to create a specific polymer. In addition to creating novel PKSs or NRPSs by combining modules, the methods of this invention can also be used to make novel modules that can add new monomeric units to a growing polypeptide or polyketide chain. Because the identity of each module, and, consequently, the identity of the monomer added by the module, is determined by the identity and number of the functional domains comprising the module, it is possible to produce novel monomeric units by creating novel combinations of functional domains within a module. Such novel modules can be created by design, for example to make a specific module that will add a specific monomer to a polyketide or polypeptide, or can be created by the random association of domains so as to produce libraries of novel modules. Such novel modules can be made using recombinant or *in vitro* synthetic means.

Mutations can be made to the native NRPS and/or PKS subunit sequences and such mutants used in place of the native sequence, so long as the mutants are able to function with other PKS and/or PKS subunits to collectively catalyze the synthesis of an identifiable polyketide and/or polypeptide. Such mutations can be made to the native sequences using conventional techniques such as by preparing synthetic oligonucleotides including the mutations and inserting the mutated sequence into the gene encoding a NRPS and/or PKS subunit using restriction endonuclease digestion. (*see, e.g.,* Kunkel, (1985) *Proc. Natl. Acad. Sci. USA* 82: 448; Geisselsoder *et al.* (1987) *BioTechniques* 5: 786). Alternatively, the mutations can be effected using a mismatched primer (generally 10-20 nucleotides in length) which hybridizes to the native nucleotide sequence, at a temperature below the melting temperature of the mismatched duplex. The primer can be made specific by keeping primer length and base composition within relatively narrow limits and by keeping the mutant base centrally located (Zoller and Smith (1983) *Meth, Enzymol.* 100: 468). Primer extension is effected using DNA polymerase, the product cloned and clones containing the mutated DNA, derived by segregation of the primer extended strand, selected. Selection can be accomplished using the mutant primer as a hybridization probe. The technique is also applicable for generating multiple point mutations (*see, e.g.,* Dalbie-McFarland *et al.* (1982) *Proc. Natl. Acad. Sci USA* 79:6409). PCR mutagenesis will also find use for effecting the desired mutations.

C) Random modification of PKS/NRPS pathways.

In another embodiment, variations can be made randomly, for example by making a library of molecular variants of a known polymer by randomly mutating one or more PKS or NRPS modules and/or enzymatic domains or by randomly replacing one or more modules or enzymatic domains in a known PKS or NRPS with a collection of alternative modules and/or enzymatic domains..

The PKS and/or NRPS modules can be combined into a single multi-modular enzyme, thereby dramatically increasing the number of possible combinations obtained using these methods. These combinations can be made using standard recombinant or nucleic acid amplification methods, for example by shuffling nucleic acid sequences encoding various modules or enzymatic domains to create novel arrangements of the sequences, analogous to DNA shuffling methods described in Crameri *et al.*, (1998) Nature 391: 288-291, and in U.S. Patents 5,605,793 and in 5,837,458. In addition, novel combinations can be made *in vitro*, for example by combinatorial synthetic methods. Novel polymers, or polymer libraries, can be screened for any specific activity using standard methods.

Random mutagenesis of the nucleotide sequences obtained as described above can be accomplished by several different techniques known in the art, such as by altering sequences within restriction endonuclease sites, inserting an oligonucleotide linker randomly into a plasmid, by irradiation with X-rays or ultraviolet light, by incorporating incorrect nucleotides during in vitro DNA synthesis, by error-prone PCR mutagenesis, by preparing synthetic mutants or by damaging plasmid DNA in vitro with chemicals. Chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, agents which damage or remove bases thereby preventing normal base-pairing such as hydrazine or formic acid, analogues of nucleotide precursors such as nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine intercalating agents such as proflavine, acriflavine, quinacrine, and the like.

Generally, plasmid DNA or DNA fragments are treated with chemicals, transformed into *E. coli* and propagated as a pool or library of mutant plasmids.

Large populations of random enzyme variants can be constructed *in vivo* using "recombination-enhanced mutagenesis." This method employs two or more pools of, for example, 10^6 mutants each of the wild-type encoding nucleotide sequence that are generated using any convenient mutagenesis technique, described more fully above, and then inserted into cloning vectors.

D) Incorporation and/or modification of non-blm cluster elements.

In either the directed or random approaches, nucleic acids encoding novel combinations of modules and/or enzymatic are introduced into a cell. In one embodiment, nucleic acids encoding one or more PKS or NRPS domains are introduced into a cell so as to replace one or more domains of an endogenous PKS or NRPS within a chromosome of the cell. Endogenous gene replacement can be accomplished using standard methods, such as homologous recombination. Nucleic acids encoding an entire PKS, NRPS, or combination thereof can also be introduced into a cell so as to enable the cell to produce the novel enzyme, and, consequently, synthesize the novel polymer. In a preferred embodiment, such nucleic acids are introduced into the cell optionally along with a number of additional genes, together called a 'gene cluster,' that influence the expression of the genes, survival of the expressing cells, *etc.* In a particularly preferred embodiment, such cells do not have any other PKS- or NRPS- encoding genes or gene clusters, thereby allowing the straightforward isolation of the polymer synthesized by the genes introduced into the cell.

Furthermore, the recombinant vector(s) can include genes from a single PKS and/or NRPS gene cluster, or may comprise hybrid replacement PKS gene clusters with, *e.g.*, a gene for one cluster replaced by the corresponding gene from another gene cluster. For example, it has been found that ACPs are readily interchangeable among different synthases without an effect on product structure. Furthermore, a given KR can recognize and reduce polyketide chains of different chain lengths. Accordingly, these genes are freely interchangeable in the constructs described herein. Thus, the replacement clusters of the present invention can be derived from any combination of PKS and/or NRPS gene sets that ultimately function to produce an identifiable polyketide and/or peptide.

Examples of hybrid replacement clusters include, but are not limited to, clusters with genes derived from two or more of the *act* gene cluster, the *whiE* gene cluster, frenolicin (*fren*), granaticin (*gra*), tetracenomycin (*tcm*), 6-methylsalicylic acid (6-msas), oxytetracycline (*otc*), tetracycline (*tet*), erythromycin (*ery*), griseusin (*gris*), nanaomycin, medermycin, daunorubicin, tylosin, carbomycin, spiramycin, avermectin, monensin, nonactin, curamycin, rifamycin and candicidin synthase gene clusters, among others. (For a discussion of various PKSs, *see, e.g.,* Hopwood and Sherman (1990) *Ann. Rev. Genet.* 24: 37-66; O'Hagan (1991) The Polyketide Metabolites, Ellis Horwood Limited.

A number of hybrid gene clusters have been constructed, having components derived from the *act, fren, tcm, gris* and *gra* gene clusters (*see, e.g.*, U.S. Patent 5,712,146). Other hybrid gene clusters, as described above, can easily be produced and screened using the disclosure herein, for the production of identifiable polyketides, polypeptides or polyketide/polypeptide hybrids.

Host cells (*e.g. Streptomyces*) can be transformed with one or more vectors, collectively encoding a functional PKS/NRPS set (*e.g.* a bleomycin or bleomycin analog), or a cocktail comprising a random assortment of PKS and/or NRPS genes, modules, active sites, or portions thereof. The vector(s) can include native or hybrid combinations of PKS and/or NRPS subunits or cocktail components, or mutants thereof. As explained above, the gene cluster need not correspond to the complete native gene cluster but need only encode the necessary PKS and/or NRPS components to catalyze the production of the desired product. For example, in *Streptomyces* aromatic PKSs, carbon chain assembly requires the products of three open reading frames (ORFs). ORF1 encodes a ketosynthase (KS) and an acyltransferase (AT) active site (KS/AT); ORF2 encodes a chain length determining factor (CLF), a protein similar to the ORF1 product but lacking the KS and AT motifs; and ORF3 encodes a discrete acyl carrier protein (ACP). Some gene clusters also code for a ketoreductase (KR) and a cyclase, involved in cyclization of the nascent polyketide backbone. However, it has been found that only the KS/AT, CLF, and ACP, need be present in order to produce an identifiable polyketide. Thus, in the case of aromatic PKSs derived from *Streptomyces*, these three genes, without the other components of the native clusters, can be included in one or more recombinant vectors, to constitute a "minimal" replacement PKS gene cluster.

E) Variation of starter and extender units.

In addition to varying the PKS and/or NRPS modules and/or domains, variations in the products produced by various PKS/NRPS systems can be obtained by varying the starter units and/or the extender units. Thus, for example, a considerable degree of variability exists for starter units, *e.g.*, acetyl CoA, maloamyl CoA, propionyl CoA, acetate, butyrate, isobutyrate and the like. In addition, naturally occurring PKSs and/or NRPSs have shown some tolerance for varying extender units.

F) Examples of preferred modifications.

As indicated above, the novel PKS and NRPS modules and enzymatic domains identified herein can be used to perform specific single modifications of particular substrates, or as components of complex synthetic pathways to generate particular products or large combinatorial libraries. As described in the Examples, a number of modules of the *blm* gene cluster provide novel functionality. By way of example, a few preferred reactions are listed below. These examples are intended to be illustrative and are not exhaustive nor limiting.

1. Use of *BlmVIII* PKS to introduce branched methyl group.

The *blmVIII* gene identified herein encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit. However, the identification of an integrated methyltransferase (MT) domain in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain. The use of this methyltransferase domain allows the introduction of a branched methyl group during a polyketide and/or polypeptide and/or hybriding polyketide/polypeptide synthesis. Figure 5 illustrates the use of *BlmVIII* PKS in engineering a polyketide biosynthesis that introduces a branched methyl group.

The first formula in Figure 5 illustrates a polyketide synthesis mediated by 6-deoxyerythronolide B synthase (DEBS) which normally catalyzes the biosynthesis of the erythromycin aglycone, 6-deoxyerythronolide B. The remaining formulas show how the use of the *blmVIII* methyltransferase (MT) group at different points in the synthesis results in the introduction of a methyl group at different locations in the resulting product.

In view of this illustration, one of skill in the art would appreciate that the *blmVIII* MT domain can be used in a wide variety of biosyntheses to introduce methyl branches.

2. Use of the *blm* gene cluster to make thiazolidine, thiazoline, thiazole, bi-thiazolidine, bithiazoline, and bithiazole-containing compounds.

The *BlmIV* and *BlmIII* NRPSs are characterized by unusual Cy domains as well an unprecedented Ox domain, providing an efficient biosynthesis for a bithiazole structure. While thiazoline is the direct product of the Cy domain, the thiazoline-to-thiazole conversion generally is performed with an additional oxidation step. We identified at the C-terminus of NRPS-0 an additional domain that shows low, but significant, sequence homology to a family of putative oxidases/dehydrogenases, including the McbC protein of the microcin B17 synthase (Table 1). Microcin B17 synthase catalyzes the synthesis of the oxazole and thiazole-containing peptide antibiotic microcin B17, and McbC has been proposed to play a role in catalyzing the oxazoline/thiazoline-to-oxazole/thiazole conversion. Consequently, we propose that this extra domain at the C-terminus of NRPS-0 provides the oxidase/dehydrogenase activity for the biosynthesis of the bithiazole moiety of BLM, defining a novel Ox domain for NRPSs.

It is noteworthy that a cell-free preparation from *Sv* ATCC15003 has been reported to catalyze the conversion of phleomycins to BLMs in the presence of NAD$^+$, supporting the hypothesis that the bithiazole moiety of BLM results from stepwise oxidations of a bithiazoline precursor (Fig. 1A). (The phleomycin producer could be imagined to result from the loss of its Ox activity for the first thiazoline ring.) Given the wide distribution of thiazole or oxazole rings in natural products exhibiting an impressive array of biological activities, the cloning of the *blmIV, III* genes and the identification of the Ox domain open many opportunities thiazole biosynthesis and to synthesize novel thiazole containing molecules by engineering peptide biosynthesis.

Representative thiazole syntheses using variants of the blm NRPS are illustrated in Figure 6. Note that in Figure 6, A^M and A^N refer to an A domain that activates and amino acid with R^M and R^N groups, respectively. A^C refers to an A domain that activates Cys (x = SH) or Ser (X = OH) that can be cyclized to form the oxiaoline/thiazoline or oxazole/thiazole structures. DH is a dehydratase. In view of these representative examples, one of skill in the art would appreciate that the *blm NRPS* domain and its variants can be used in a wide variety of chemical syntheses make thiazolidine, thiazoline, thiazole, bi-thiazolidine, bithiazoline, or bithiazole-containing compounds.

3. Use of the *blm* gene cluster to make heterocyclic ring-containing compounds.

Various *blm* modules can be used to produce heterocyclic ring-containing compounds. Such heterocycles include, but are not limited to five member S- and N-containg compounds of the thiazolidine, thiazoline and thiazole family or the O- and N-containing compounds of the oxazolidine, oxazoline, and oxazole family. Again, the preparation of such compounds is illustrated in Figure 6.

4. Use of the *blm* gene cluster to make sugars.

In still another embodiment, the *blm* gene cluster or elements thereof can be used to make sugars. Such sugars include, but are not limited to L-sugars (with the *BlmG* epimerase), sugars modified by a carbamoyl group (*e.g.,* using *BlmD*), and various disaccharides. Representative examples of such syntheses are illustrated in Figure 7. Such sugar biosynthesis genes can also e used to attach sugars onto other polyketide and/or peptide aglycones.

F) Screening of products.

Particularly where large combinatorial libraries are synthesized, *e.g.* using one or more modules and/or enzymatic domains of the *blm* gene cluster it will often be desired to screen the resulting compound(s) for the desired activity. Mehtods of screening compounds (*e.g.* polypeptides, polyketides, sugars, thiazoles, *etc.*) for various activities of interest (*e.g.* cytotoxicity, antimicrobial activity, particular chemical activities, *etc.*) are well known to those of skill in the art.

Where large numbers of compounds are produced, it is often desired to rapidly screen such compounds using "high throughput systems" (HTS). High throughput assays systems are well known to those of skill in the art and many such systems are commercially available. (*see, e.g.*, Zymark Corp., Hopkinton, MA; Air Technical Industries, Mentor, OH; Beckman Instruments, Inc. Fullerton, CA; Precision Systems, Inc., Natick, MA, etc.). These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughputand rapid start up as well as a high degree of flexibility and customization. The manufacturers of such systems typically provide detailed protocols for the various high throughput screens.

VII. *In Vitro* syntheses.

In additional embodiments of this invention, bleomycins and other polyketides and/or polypeptides are synthesized and/or modified *in vitro*. Individual enzymatic domains or modules can be used *in vitro* to modify a unit and/or to add a single monomeric unit to a growing polyketide or polypeptide chain. In one approach a metasynthetase providing all the desired synthetic activities recombinantly expressed and then provided, the appropriate substrates and buffer system *e.g.* in a bioreactor, to direct the synthesis of the desired product. In another approach, various PKSs and/or NRPSs are provided in different solutions and the growing polymer chains can be sequentially introduced into the plurality of solutions, each containing a single (or several) PKS or NRPS modules. In still another embodiment, the PKS and/or NRPS modules or enzymatic domains are provided attached to a solid support and a fluid contgaining the growing macromolecule is passed over the surface whereby the PKSs or NRPSs are able to react with the target substrate.

In one preferred embodiment, a combinatorial library of polyketides or polypeptides, or combinations thereof, is created by using automated means to facilitate the sequential introduction of a multitude of polymeric chains, each attached to a solid support, to a collection of solutions, each containing a single PKS or NRPS module. These automated means can be used to systematically vary the sequence by which each polymeric chain is introduced into the various solutions, thereby creating a combinatorial library. Numerous methods are well known in the art to create combinatorial libraries of molecules by the sequential addition of monomeric units, for example as described in WO 97/02358.

VIII. Kits.

In still another embodiment, this invention provides kits for practice of the methods described herein. In one preferred embodiment, the kits comprise one or more containers containing nucleic acids encoding one or more of the *blm* gene cluster ORFs and/or one or more of the BLM PKS or NRPS modules or enzymatic domains. Certain kits may comprise vectors encoding the *blm* orfs and/or cells containing such vectors. The kits may optionally include any reagents and/or apparatus to facilitate practice of the assays described herein. Such reagents include, but are not limited to buffers, labels, labeled antibodies, bioreactors, cells, *etc.*

In addition, the kits may include instructional materials containing directions (*i.e.*, protocols) for the practice of the methods of this invention. Preferred instructional materials provide protocols utilizing the kit contents for creating or modifying *blm* module or ORF and/or for synthesizing or modifying a molecule using one or more *blm* modules and/or enzymatic domains. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1

Bleomycin biosynthesis in *Streptomyces verticillus* ATCC15003, A model for hybrid peptide and polyketide biosynthesis.

Here we report the cloning and characterization of the *blm* biosynthesis gene cluster from *Sv* ATCC15003 (Fig. 2). Sequence analysis and biochemical characterization of individual modules enabled us to align the nine NRPS and one PKS modules in a linear order to constitute the Blm megasynthetase complex (Fig. 1B). These studies revealed several unprecedented features for peptide and polyketide biosynthesis, setting the stage to investigate the molecular basis for intermodular communication between NRPS and PKS, and supported the wisdom of combining individual NRPS and PKS modules for combinatorial biosynthesis to make novel "unnatural" natural products from amino acids and short carboxylic acids.

Materials and Methods.

General procedures.

Escherichia coli DH5α (Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual,* 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA), *E. coli* XL 1-Blue MR (Stratagene, La Jolla, CA), *E. coli* BL21(DE-3) (Novagen, Madison, WI), and *Sv* ATCC15003 (American Type Culture Collection, Rockville, MD) were used in this work. pOJ446 (Agricultural Research Service Culture Collection, Peoria, IL), pQE60 (Qiagen, Santa Clarita, CA), pET28a and pET29a (Novagen), and other plasmids were from commercial sources. *E. coli* (Sambrook, *supra.*) and *Sv* ATCC15003 strains (Hopwood *et al.* (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich, UK) were cultured under standard conditions.

Plasmid preparation was carried out by using commercial kits (Qiagen). Total *Sv* ATCC15003 DNA was isolated according to literature protocols (Hopwood *et al.* (1985) *Genetic Manipulation of Streptomyces: A Laboratory Manual*, The John Innes Foundation, Norwich, UK; Nagaraja *et al.* (1987) *Methods Enzymol.* 153: 166-198). Restriction enzymes and other molecular biology reagents were from commercial sources, and digestions and ligation followed standard methods (Sambrook, *supra.*). For Southern analysis, digoxigenin labelling of DNA probes, hybridization, and detection were performed according to the protocols provided by the manufacturer (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Automated DNA sequencing was carried out on an ABI Prism 377 DNA Sequencer (Perkin-Elmer/ABI, Foster City, CA), and this service was provided by either the DBS Automated DNA Sequencing Facility, UC Davis, or Davis Sequencing (Davis, CA). Data were analyzed by the ABI Prism Sequencing 2.1.1 software and the Genetics Computer Group (GCG) program (Madison, WI).

<u>Cloning and sequencing of the *blm* gene cluster.</u>

A genomic library of *Sv* ATCC15003 was constructed in pOJ446 according to literature procedures (Nagaraja *et al.* (1987) *Methods Enzymol.* 153: 166-198) and screened with probes made from both ends of the *blmAB* locus (Sugiyama *et al.* (1994) *Gene* 151: 11-16; Calcutt and Schmidt (1994) *Gene* 151: 17-21), leading to the localization of 140-kb contiguous DNA, of which 100-kb is upstream (Fig. 2) and 40-kb is downstream (data not shown) of the *blmAB* genes. Heterologous NRPS probes were amplified from *Sv* ATCC15003 by polymerase chain reaction (PCR) according to literature procedures (Turgay and Marahiel (1994) *Peptide Res.* 7: 238-241) and used to screen the entire 140-kb DNA by Southern analysis under various hybridization conditions (Shen *et al.* (1999) *Bioorg. Chem.* 27: 155-171).

<u>Prediction of substrate specificity of NRPSs.</u>

The nine Blm NRPS modules were compared with eighty four modules from various bacterial and fungal NRPSs available at the GenBank, including those with known or putative specificity for amino acids present in BLM. A table of overall similarities/identities was generated by PILEUP analysis of the A3 to A6 regions, and the residues lining the substrate binding pocket by comparison with PheA (Conti *et al.* (1997) *EMBO J.* 16, 4174-4183) were determined by PILEUP/PRETTY analysis. The percentage similarities for each Blm NRPS module were plotted against the rest of the NRPS modules to display the overall sequence homology between the A3 to A6 region. Those modules that showed significantly higher homology were selected to compare the amino acid residues that line the substrate binding pocket.

Overproduction and biochemical characterization of the NRPS-1A and NRPS-6A proteins.

Heterologous expression of the A domain in *E. coli* were performed according to literature procedures (Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850). NRPS-1A (forward primer 5'-AAC CCA TGG CTG CTT CCC TGA CCC GCC TGG CC-3', SEQ ID NO:76, and reverse primer 5'-CCT AGA TCT ACG GGC AGG TGG GGC GGT-3', SEQ ID NO:77) and NRPS-6A (forward primer 5'-GGG AAT TCC ATA TGA TCC TCA CGT CCT TCC AC-3', SEQ ID NO:78, and reverse primer 5'-GGC AAG CTT GGG TGA GGG TCC GTT CGG T-3', SEQ ID NO:79) were amplified by PCR from *Sv* ATCC15003 cosmid clones. The resulting 1.6-kb fragment of NRPS-1A was first cloned into the *Nco*I/*Bgl*II sites of pQE60 and then moved as an *Nco*I/*Hind*III fragment into the similar sites of pET29a to yield pBS10, and the resulting 1.6-kb fragment of NRPS-6A was directly cloned into the *Nde*I/*Hind*III sites of pET28a to yield pBS11. Introduction of pBS10 and pBS11 into *E. coli* BL21(DE-3) under standard expression conditions resulted in production of NRPS-1A (with an N-terminal S-tag and a C-terminal His$_6$-tag) and NRPS-6A (with an N-terminal His$_6$-tag), respectively. The soluble fractions of fusion proteins were subjected sequentially to an affinity chromatography on Ni-NTA resin and an anion exchange chromatography on a Hyper-D column (PerSeptive Biosystem, Framingham, MA), resulting in NRPS-1A and NRPS-6A with near homogeneity.

Results and Discussion.

Cloning of the *blm* gene cluster from *Sv* ATCC15003.

Davies and co-workers previously cloned two BLM resistance genes (*blmA* and *blmB*) from *Sv* ATCC15003 (Sugiyama *et al.* (1994) *Gene* 151: 11-16), and Calcutt and Schmidt (1994) *Gene*, 151: 17-21, sequenced a 7.2-kb DNA fragment flanking the *blmAB* genes, revealing seven open reading frames (orfs), none of which were found to encode Blm NRPS or PKS enzymes. Given the precedent that antibiotic production genes commonly occur as a cluster in actinomycetes, we adopted an approach combining chromosomal walking from the *blmAB* resistance locus and DNA hybridization with heterologous NRPS probes to clone and identify the *blm* cluster, leading to the localization of 140-kb contiguous *Sv* ATCC15003 DNA. DNA sequencing of approximately 90-kb of the *blm* gene cluster, including the 7.2-kb *blmAB* locus, revealed 40 ORFs (Fig. 2). Preliminary functional assignments were made by comparison of the deduced gene products with proteins of known functions in the database. Among the ORFs identified from the *blm* cluster, we indeed found a PKS module, flanked by several NRPS modules–a fact that supports the hybrid NRPS/PKS/NRPS hypothesis for BLM biosynthesis–along with several sugar biosynthesis genes and genes encoding other biosynthesis enzymes as well as several resistance and regulatory genes (Table 1).

Noteworthy are the genes encoding the putative NRPS and PKS enzymes. The *blmI, blmII,* and *blmXI* genes encode NRPSs with an unusual architecture. In contrast to all known NRPSs, which are of modular organization with each module consisting minimally of a condensation (C), an adenylation (A), and a peptidyl carrier protein (PCP) domain (1), BlmI, BlmII, and BlmXI are discrete proteins homologous to individual domains of type I NRPSs. We have characterized BlmI as a type II PCP (18). The BlmII and BlmXI proteins could serve as candidates for type II condensation enzymes. It is unclear yet what role if any these discrete NRPS enzymes could play in BLM biosynthesis.

The *blmIII, blmIV, blmV, blmVI, blmVII, blmIX*, and *blmX* genes encode modular NRPSs consisting of domains characteristic for known type I NRPSs (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706), such as the A, PCP, C, and condensation/cyclization (Cy) domains (Konz *et al.* (1997) *Chem. Biol.* 4: 927-937), as well as an unprecedented oxidation (Ox) domain (see discussion below). However, BlmVI is unique among all the Blm NRPSs identified. Its N-terminal module (NRPS-5) consists of an atypical A domain, which bears a close resemblance to a family of acyl CoA synthases (Fitzmaurice and Kolattukudy (1997) *J. Bacteriol.* 179: 2608-2615; Fitzmaurice and Kolattukudy (1998) *J. Biol. Chem.* 273: 8033-8039), and an acyl carrier protein (ACP)-like domain (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706). Its C-terminal module is truncated and presumably interacts with BlmV to constitute the complete NRPS-3 module (Fig. 1B). Also noteworthy are the C domain of NRPS-3 that lacks both

His residues of the conserved HHxxxDG (SEQ ID NO:4) active site for transpeptidation (Stachelhaus *et al.* (1998) *J. Biol. Chem.,* 273: 22773-22781) and the extra C domain at the C-terminus of BlmV. These unusual features associated with *BlmVI* and *BlmV* may play roles in the formation of the β-aminoalaninamide and the pyrimidine moieties of BLM, which are unprecedented in peptide biosynthesis. For example, we propose that the NRPS-4-activated Ser is first dehydrated into dehydroalanine before condensation–an analogous Thr-to-2,3-dehydroaminobutyric acid dehydration has been observed in syringomycin biosynthesis (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863). Conjugate addition to dehydroalanine by Asn on the NRPS-3 module downstream followed by an aminolysis to cleave the Ser-Asn adduct off the Blm megasynthetase furnishes the β-aminoalaninamide moiety (Fig. 1B). The former reaction could be catalyzed by the C domain of NRPS-3 that apparently is nonfunctional for normal transpeptidation due to the lack of the active sites, and the latter reaction could be catalyzed by the acyl CoA synthase-like domain of NRPS-5 in a process that resembles the acyl CoA synthase-catalyzed synthesis of acyl CoA from carboxylic acid (Stachelhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781; Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863) but in the reverse direction in the presence of an amino donor (Fig. 1B).

The *blmVIII* gene encodes a PKS module consisting of domains characteristic for known PKSs, such as ketoacyl synthase (KS), acyltransferase (AT), ketoreductase (KR), and ACP, with malonyl CoA acting as an extending unit according to sequence comparison of the AT domain (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) (Fig. 1B). However, the identification of an integrated methyltransferase (MT) domain (Kagan and Clarke (1994) *Arch. Biochem. Biophys.* 310: 417-427) in the middle of *BlmVIII* is unique, representing the first PKS from actinomycetes that contains an internal MT domain. The only other example of PKS from bacteria that contains an internal MT domain is HMWP1 of the yersiniabactin gene cluster (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546). It has been assumed that fungal PKSs in general contain internal MTs for the introduction of methyl branch into the polyketide products, as it has been shown recently in lovastatin biosynthesis (Kennedy *et al.* (1999) *Science* 284: 1368-1372).

The Blm megasynthetase-templated assembly of BLM.

According to the hybrid NRPS/PKS/NRPS model for BLM biosynthesis (Fig. 1A), we predict a linear modular organization of individual NRPS and PKS modules to constitute the Blm megasynthetase. Thus, the first functional domain of the Blm megasynthetase should be a NRPS module that initiates BLM biosynthesis by activating L-Ser as an amino acylthioester to set the stage for transpeptidation. Chain elongation proceeds by sequential incorporation of L-Asn, L-Asn, L-His, and L-Ala, requiring four additional NRPS modules. In the next step, a malonate reacts with the resulting pentapeptide intermediate to form a β-ketothioester intermediate that is subsequently methylated at the α-position and reduced at the β-keto group. A PKS module presumably dictates all these biosynthetic events and interacts with the aligned NRPS module upstream to channel the growing peptide intermediate from an NRPS module to a PKS module. After one cycle of polyketide elongation, peptide elongation is resumed by incorporation of an L-Thr residue. This step is presumably catalyzed by an NRPS module that interacts with the upstream PKS module to channel the growing polyketide intermediate (as far as the active site is concerned) from a PKS module to an NRPS module. At this stage, methylation occurs at the pyrimidine moiety of the growing intermediate, presumably catalyzed by a discrete methyltransferase; chain elongation is continued by three additional NRPS modules that incorporate a β-Ala and two L-Cys molecules sequentially. Finally, the fully assembled BLM peptide/polyketide/peptide backbone is hydroxylated at the β-position of the His residue, presumably by a discrete hydroxylase, and released from the Blm megasynthetase complex via nucleophilic substitution of the RCO-S-PCP species by a terminal amine to form the BLM aglycone. Intermediates after five of the nine proposed elongation steps were in fact isolated as P-3, P-3A, P-3K, P-4, P-5, P-5m, P-6m, and P-6mo (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), which presumably resulted from premature departure from the Blm megasynthetase complex before the chain reaches its full length (Fig. 1B).

Most of the bacterial NRPS gene clusters characterized to date are organized in operon-type structures, encoding multimodular NRPS proteins with individual modules organized along the chromosome in a linear order that parallels the order of the amino acids in the resultant peptides, i.e., following the "colinearity rule" for the NRPS-templated assembly of peptides from amino acids (A special thematic issue on polyketide and nonribosomal polypeptide biosynthesis, (1997) *Chem. Rev.* 97: 2463-2706; Cane *et al.* (1998) *Science* 282: 63-68). Inspection of the *blm* gene cluster (Fig. 2) showed that the Blm NRPS and PKS modules apparently are not organized according to the "colinearity rule" for BLM biosynthesis (Fig. 1). [Exception to the "colinearity rule" was also noted in the syringomycin synthetase gene cluster (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 32857-32863), and in fact, Grandi and co-workers have demonstrated recently in *Bacillus subtilis* that neither the operon-type structure nor the physical linkage of individual modules is essential for proper assembly and activity of the surfactin NRPS megasynthetase (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 14403-14410).] Realizing that the BLM biosynthesis cannot be rationalized according to the "colinearity rule", we determined the substrate specificity of individual NRPS and PKS modules in an attempt to shed light on the modular organization of the Blm megasynthetase complex. Brick and co-workers postulated, based on the X-ray structural analysis of the A domain of GrsA, PheA, that the region between core sequences A3 to A6 represent the amino acid specificity determinant of an NRPS module (Conti *et al.* (1997) *EMBO J.* 16: 4174-4183). Since the A domains in all known NRPSs share a significant sequence identity (ensuring that the main chain conformation of the enzymes is likely to be very similar), they further proposed that the differing substrate specificity of individual NRPS modules will be mainly determined by the nature of the amino acids lining the substrate binding pocket (Stachelhaus *et al.* (1999) *Chem. Biol.* 6: 493-505; Conti *et al.* (1997) *EMBO J.* 16: 4174-4183). Given this structural information and the vast amount of NRPS sequences available at the GenBank, we developed a novel approach for predicting substrate specificity for NRPS modules by comparing the overall sequence between the A3 to A6 region and the eight amino acid residues that line up the substrate binding pocket. While a constant level of similarities (30%-40%) was evident among all the NRPS modules analyzed, most of the Blm NRPS modules showed striking similarities (50%-60%) to a particular cluster of NRPS modules as exemplified in Fig. 3A for NRPS-1 and NRPS-6. Close examination of these modules clustered with higher similarities revealed that they activate the same or very similar amino acid, based on which the putative substrate for the NRPS in query could be predicted, i.e., NRPS-1 and NRPS-6A activate L-Cys and L-Thr, respectively. These predictions were further supported by comparing the residues lining the substrate binding pocket. For example, the amino acid residues lining the substrate binding pocket for NRPS-1 and NRPS-6 are almost identical to those NRPS modules that are known to activate L-Cys and L-Thr, respectively, as shown in Fig. 3B. To verify the predicted amino acid specificity, we overproduced and purified the NRPS-1A and NRPS-6A proteins (Fig. 3C) and examined their substrate specificity according to the amino acid-dependent ATP-PPi assay (Lee *et al.* (1970 *Meth. Enzymol.,* 43: 585-602; Ku *et al.* (1997) *Chem. & Biol.,* 4: 203-207). NRPS-1A and NRPS-6A indeed activate specifically L-Cys and L-Thr, respectively, among the amino acids tested (Fig. 3D). The latter results greatly enhanced our confidence in predicting the substrate specificity of a NRPS module by the above method. We subsequently determined the substrate specificity for all the NRPS modules identified from the *blm* gene cluster and they in fact accounted for all nine amino acids required for BLM biosynthesis (Fig. 2).

Using the substrate specificity of individual NRPS and PKS modules as a guide, we can align the nine NRPS and one PKS modules to constitute the Blm megasynthetase as shown in Fig. 1B according to our hybrid NRPS/PKS/NRPS model for BLM biosynthesis (Fig. 1A). Among all the PKSs or NRPS systems examined so far, the Blm megasynthetase consists of the largest number of individual proteins. The precise interactions among all the Blm NRPS and Blm PKS proteins to constitute the Blm megasynthetase complex, therefore, reflect a remarkable power of protein-protein recognition (Guenzi *et al.* (1998) *J. Biol. Chem.* 273: 14403-14410; Gokhale *et al.* (1999) *Science* 284: 482-485). Although we are yet to provide direct evidence supporting the specific protein-protein interactions between the neighboring proteins, it is striking to note that all the biosynthetic intermediates isolated are derailed from either PKS or NRPS modules at the junctions between the interacting proteins (Fig. 1B). Since it is not difficult to imagine that an intermediate is more likely to fall off the enzyme complex when it is subjected to interpeptide transfer than to intrapeptide transfer, we view the latter observation as strong evidence supporting the current model of the Blm megasynthetase

**BlmIX/BlmVIII/BlmVII as a hybrid NRPS/PKS/NRPS model. **

Recent biosynthetic studies on rapamycin in *Streptomyces hygroscopicus* (Konig *et al.* (1997) *Eur. J. Biochem.* 247: 526-534), yersiniabactin in *Yersinia enterocolitica* and *Y. pestis* (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650) and TA in *Myxococcus xanthus* (Paitan *et al.* (1999) *J. Mol. Biol.* 286, 465-474) are starting to shed light on hybrid peptide and polyketide biosynthesis. Two models are emerging for the alignment between a NRPS and a PKS module. The interacting NRPS and PKS modules could be either covalently linked by arranging all domains in a linear order on the same protein (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474) or physically located on two separate proteins, requiring specific protein-protein recognition to ensure the correct pairing between the interacting modules (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546; Konig *et al.* (1997) *Eur. J. Biochem.* 247: 526-534;

Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). Common to all these systems, however, are the unusual features associated with the interacting modules, such as the lack of the AT domain of the PKS module in Ta1 (Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474) and the lack of the A domain and the presence of the Cy domain of the NRPS modules in both HMWP1 and HMWP2 (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-5461; Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). While extremely intriguing, the latter features complicate mechanistic analysis of these systems, making them less ideal candidates for studying how NRPS and PKS integrate into a productive hybrid NRPS/PKS complex.

The *BlmIX/BlmVIII/BlmVII* system combines the features of both hybrid NRPS/PKS and PKS/NRPS systems, serving as an ideal model for studying hybrid peptide and polyketide biosynthesis. The fact that both the *BlmIX* and *BlmVII NRPS* modules and the *BlmVIII* PKS module themselves are three separate proteins with a typical domain organization for NRPS and PKS enzymes greatly simplifies the mechanistic analysis of the hybrid NRPS/PKS/NRPS complex. We have found that the KS domain of BlmVIII is more similar to the KSs of HMWP1 (Pelludat *et al.* (1998) *J. Bacteriol.* 180: 538-546) and Ta1 (Paitan *et al.* (1999) *J. Mol. Biol.* 286: 465-474), both of which catalyze the elongation of a peptidyl intermediate with a malonate, than to KSs of type I PKSs. We attribute these subtle differences to their unique reactivity that catalyzes the transfer of the peptidyl intermediate from the PCP to the KS domain, which presumably takes place prior to chain elongation (Fig.4). Subsequent condensation catalyzed by the KS domain between the peptidyl intermediate and malonyl-S-ACP results in the elongation of the growing peptide with a carboxylic acid. Equally striking are the discoveries that the ACP domain of BlmVIII is more similar to a PCP than to an ACP and that the C domain of BlmVII has an additional N-terminal segment of about 50 amino acids that is rich in arginine, aspartic acid, and glutamic acid. The latter feature is analogous to the N-terminal interpolypeptide linker for type I PKS, which has recently been demonstrated to play a critical role in intermodular communication (Gokhale *et al.* (1999) *Science* 284: 482-485). We propose that these unique features of the ACP domain from the BlmVIII PKS module and the C domain from the BlmVII NRPS module provide the molecular basis for the C domain to recognize the acyl-S-ACP as a substrate. Subsequent condensation catalyzed by the C domain between acyl-S-ACP and amino acyl-S-PCP results in the elongation of the growing polyketide (as far as this condensation is concerned) with an amino acid (Fig. 4).

Novel domains for the Blm NRPS and PKS modules.

Various NRPS and PKS domains have been characterized, which are the building blocks for the entire field of combinatorial biosynthesis. The success for combinatorial biosynthesis depends critically upon the repertoire of these individual domains. Genetic analysis of the *blm* gene cluster has uncovered several novel NRPS and PKS domains. Without being bound to a particular theory, it is believed that *BlmVI* and *BlmV* are involved in the biosynthesis of the β-aminoalaninamide and pyrimidine moieties of BLM). In addition, the MT domain in BlmVIII, the Cy domains in BlmIV, and the Ox domain in BlmIII are novel domains.

The BlmVIII PKS module apparently furnishes the "propionate" unit into BLM in two steps by evolving a malonyl CoA-specifying AT domain coupled with a novel S-adenosylmethionine-requiring MT domain, representing a new mechanism to introduce methyl branches into polyketides (Fig. 4). This biosynthetic reaction sequence is unprecedented for polyketide biosynthesis since all PKSs from actinomycetes examined to date incorporate the alkyl branches into the resultant polyketides by selecting various alkyl malonates as the extending units that are determined by the AT domains. Yet, feeding experiments have unambiguously established that the polyketide moiety of BLM was derived from an acetate and a methionine (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), a fact that fits well with the observed unusual domain organization of the BlmVIII PKS module (Fig. 4). It is conceivable that the combination of this MT domain with an AT domain specific for a methyl malonate extending unit (Haydock *et al.* (1995) *FEBS Lett.* 374: 246-248) could result in the synthesis of polyketides with a gem-dimethyl moiety via engineering polyketide biosynthesis. Such a gem-dimethyl group has been found to be a very important pharmacophore for the epothilones, a family of hybrid peptide and polyketide metabolites that exhibits a remarkable antitumor activity similar to taxol (Ojima *et alo.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 4256-4261).

The BlmIV and BlmIII NRPSs are characterized by the unusual Cy domains as well as the unprecedented Ox domain, providing an efficient biosynthesis for a bithiazole structure. The Cy domain was first defined by Marahiel and co-workers in their study of bacitracin biosynthesis in *B. licheniformis* (Konz *et al.* (1997) *Chem. Biol.* 4: 927-937), and the Cy activity was demonstrated recently by Walsh and co-workers in their study of the

HMWP1 and HMWP2 proteins for yersiniabactin biosynthesis in *Y. pestis* (Gehring *et al.* (1998) *Chem. Biol.* 5: 573-586; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650). While thiazoline is the direct product of the Cy domain, the thiazoline-to-thiazole conversion requires an additional oxidation step. We identified at the C-terminus of NRPS-0 an additional domain that shows low, but significant, sequence homology to a family of putative oxidases/dehydrogenases, including the McbC protein of the microcin B17 synthase (Table 1). Microcin B17 synthase catalyzes the synthesis of the oxazole and thiazole-containing peptide antibiotic microcin B17, and McbC has been proposed to play a role in catalyzing the oxazoline/thiazoline-to-oxazole/thiazole conversion (Li *et al.* (1996) *Science* 274: 1188-1193; Milne, *et al.* (1999) *Biochemistry* 38: 4768-4781). Consequently, we propose that this extra domain at the C-terminus of NRPS-0 could provide the oxidase/dehydrogenase activity needed for the biosynthesis of the bithiazole moiety of BLM, defining a novel Ox domain for NRPSs. It is noteworthy that a cell-free preparation from *Sv* ATCC15003 has been reported to catalyze the conversion of phleomycins to BLMs in the presence of NAD$^+$ (Takita and Muroka (1990) pages 289-309 in *Biochemistry of Peptide Antibiotics: Recent Advances in the Biotechnology of β-Lactams and Microbial Peptides*, Kleinkauf, H. & von Döhren, H. eds., W. de Gruyter, N.Y.), supporting the hypothesis that the bithiazole moiety of BLM results from stepwise oxidations of a bithiazoline precursor (Fig. 1A). (The phleomycin producer could be imagined to result from the loss of its Ox activity for the first thiazoline ring.) Given the wide distribution of thiazole or oxazole rings in natural products (Ojima *et alo.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 4256-4261; Li *et al.* (1996) *Science* 274: 1188-1193) exhibiting an impressive array of biological activities, the cloning of the *blmIV,III* genes and the identification of the Ox domain open many opportunities to define the mechanism for thiazole biosynthesis and to potentially synthesize novel thiazole containing molecules by engineering peptide biosynthesis.

Example 2

Identification and characterization of a type II peptidyl carrier protein from the bleomycin producer *Streptomyces verticillus* ATCC 15003.

Results.

Cloning and sequence analysis of the *blmI* gene

In our effort to clone the gene cluster responsible for BLM biosynthesis, we have determined 80 kb DNA sequence from *Sv* ATCC15003 (Fig. 8). Among the orfs identified within the *blm* gene cluster is the small orf of 273 base pairs (bp), *blmI*, which is located approximately 4 kb upstream of the previously characterized *blmAB* resistance locus (Sugiyama *et al.* (1994) *Gene* 151: 11-16; Calcutt and Schmidt (1994) *Gene* 151: 17-21) (Fig. 8B). The *blmI* gene encodes a protein of 90 amino acids with a molecular weight of 9957 and a pI of 6.52 (Fig. 8C). Computer-assisted analysis (Altschul *et al.* (1997) *Nucleic Acids Res.* 25: 3389-3402) of the deduced amino acid sequence indicates that BlmI is very similar to various PCP domains of NRPSs (ranging around 40% identity and 60% similarity, as shown in Figure 9). Like known PCP domains of NRPS, BlmI has the highly conserved signature motif of LGGXS, within which the serine residue is the site for 4'-phosphopantetheinylation (Stachelhaus and Marahiel (1995) *FEMS Microbiol. Lett.* 125: 3-14; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673). The latter posttranslational modification is generally necessary for peptide biosynthesis; converting the apo-PCP into the functional holo-PCP (Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315). Based on sequence comparison, *BlmI* is most related to PCPs and not to other kinds of carrier proteins that also share the same LGGXS (SEQ ID NO:80) motif and undergo the same posttranslational 4'-phosphopantetheinylation [31], such as the *E. coli* acyl carrier protein (ACP) (Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661), the ACP domain of type I PKS and the type II PKS ACP (Cox and Simpson (1997) *FEBS Lett.* 405: 267-272; Carreras *et al.* (1997) *Biochemistry* 36: 11757-11761), the ArCP domain (Gehring *et al.* (1998) *Biochemistry* 37: 2648-2659), and several nodulation related ACP-like proteins (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954; Spaink *et al.* (1991) *Nature* 354: 125-130).

Overexpression of *blmI* in *E. coli*

To overexpress the *blmI* gene in *E. coli*, we directly amplified the *blmI* gene by PCR from the *Sv.* ATCC15003 genomic DNA and cloned it into the pQE-60 expression vector to give pBS1 so that BlmI could be produced as a protein with a native N-terminus and a His_6-tag at its C-terminus. However, no production of the BlmI protein was detected, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), upon introduction of pBS1 into *E. coli* M15(pREP4) under the standard overexpression conditions recommended by the manufacturer (Qiagen). We reasoned that the small BlmI protein with its native N-terminus may not be stable in the heterologous host, and hence moved the *blmI* gene from pBS1 into pET-29a to yield the second overexpression construct of pBS2. In the latter construct, BlmI should be produced as a fusion protein with 27 extra amino acid residues at its N-terminus, including an S-tag and the thrombin cleaving site, in addition to the His_6-tag at its C-terminus. Introduction of pBS2 into *E. coli* BL21(DE-3) under the standard overexpression conditions recommended by the manufacturer (Novagen) indeed resulted in overproduction of BlmI. In fact, the bulk of the soluble protein was the overproduced BlmI, which was easily purified by affinity chromatography using Ni-NTA resin (Qiagen). It is noteworthy that fusion of the additional 23 amino acids to the N-terminus of BlmI as in pBS2 and change of the expression system from *E. coli* M15(pREP4) (pBS1) to *E. coli* BL21(DE-3)(pBS2) dramatically improved the expression level of *blmI.*

In vivo 4'-phosphopantetheinylation of the BlmI protein

To establish BlmI as a type II PCP, we tested if it could serve as a substrate for a PCP-specific 4'- PPTase. PPTases catalyze the posttranslational modification of an apo-PCP into a holo-PCP by transferring the 4'-phosphopantetheine moiety from co-enzyme A (CoA) to the conserved serine residue of PCP, and this reaction has been developed recently into a general method to prepare various holo-PCP, holo-ACP, or holo-ArCP from the corresponding apoproteins (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-9211; Gehring *et al.* (1998) *Biochemistry* 37: 2648-2659; Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). Therefore, we decided to investigate the 4'-phosphopantetheinylation of BlmI under both *in vivo* (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) and *in vitro* (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595) conditions.

To examine 4'-phosphopantetheinylation of BlmI in vivo, we chose *E. coli* OG7001 as the expression host, which is a β-alanine auxotroph derived from *E. coli* BL21(DE3) by P1 co-transduction of the *panD* mutation from *E. coli* SJ16 (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954). Upon introduction of pBS2 into *E. coli* OG7001, *blmI* was exceptionally well expressed and the overproduced BlmI protein was readily purified. However, high performance liquid chromatography (HPLC) analysis showed that the purified BlmI was essentially in the apo-form (Fig. 10A), indicative that apo-BlmI was a poor substrate for the *E. coli* endogenous PPTases, such as EntD and ACP synthase (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315; Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661). To circumvent the poor endogenous PPTase activity, we next co-expressed *blmI* with the *gsp* gene, which was isolated from the gramicidin S producer *Bacillus brevis*, and encoded a PPTase that was known to 4'-phosphopantetheinylate heterologously produced PCPs in *E. coli* (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207). We co-transformed pDPT-Gsp, in which the expression of the *gsp* gene was under the control of the T5/Lac promoter (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207), and pBS2 into *E. coli* OG7001. *BlmI* was again very well expressed and the resulting BlmI protein was similarly purified. HPLC analysis showed that at least 60% of overproduced BlmI was modified into the holo-BlmI protein (Fig. 10B). (A PCP domain was similarly 4'-phosphopantetheinylated in vivo before by co-expressing *gsp* in *E. coli* using pDPT-Gsp, and approximately 80% of the PCP was produced in the holo-form (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207).

We next cultured *E. coli* OG7001(pBS2) and *E. coli* OG7001(pBS2/pDPT-Gsp) in the presence of [3-^{3}H]-β-alanine, a known biosynthetic precursor of 4'-phosphopantetheine (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954). Specific incorporation of [3-^{3}H]-β-alanine into the 4'-phosphopantetheine moiety of holo-BlmI was determined by autoradiographic analysis. Thus, while fermentation of *E. coli* OG7001(pBS2) in the presence of [3-^{3}H]-β-alanine led to an IPTG-dependent overproduction of BlmI, little of the resulting BlmI protein was ^{3}H-labeled, indicative of being produced in the apo-form. In contrast, fermentation of *E. coli* OG7001(pBS2/pDPT-Gsp) in the presence of [3-^{3}H]-β-alanine resulted in a significant increase of IPTG-dependent incorporation of the ^{3}H-label into the overproduced BlmI protein, suggesting a specific incorporation of [3-^{3}H]-β-alanine into holo-BlmI, presumably in the 4'-phosphopanthetheine moiety. There were several additional proteins that were also weakly labeled by [3-^{3}H]-β-alanine. However, both their expression and their incorporation by ^{3}H-label were independent from either IPTG induction or the presence of Gsp, hence these proteins were unrelated to BlmI. (Similar background labeling was reported before for in vivo 4'-phosphopanthetheinylation of other PCP (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954)). We also purified the BlmI protein from *E. coli* OG7001(pBS2/pDPT-Gsp) and demonstrated that it was the holo-BlmI protein that was specifically associated with the ^{3}H-activity. Finally, we confirmed the identity of holo-BlmI by subjecting the purified BlmI protein to MALDI-Tof mass spectral analysis (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). BlmI produced in the absence of the Gsp PPTase yielded a single peak with a molecular weight of 13,952, suggesting that the produced BlmI protein is in the apo-form (calc., 13,949). In contrast, BlmI produced in the presence of Gsp yielded two species with molecular weight of 13,969 and 14,303, respectively. While the species with the molecular weight of 13,969 represents apo-BlmI, a molecular weight of 14,303 unambiguously confirmed the other protein as holo-BlmI (calc., 14,289). The latter result indicated that the purified BlmI consisted of both the apo- and holo-BlmI proteins, in agreement with the HPLC analysis results (Fig. 10B).

In vitro 4'-phosphopantetheinylation of the BlmI protein

To investigate 4'-phosphopantetheinylation of BlmI in vitro, we chose the Sfp protein as the preferred PPTase, which had been isolated before from the surfactin producer *Bacillus subtilis* (Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321). (Overexpression of *gsp* in *E. coli* using pDPT-Gsp resulted in predominantly an insoluble Gsp protein (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207). The Sfp PPTase was overproduced in *E. coli* MV1190(pUC8-Sfp) and purified to near homogeneity as described before (Quadri *et al.* (1998) *Biochem.,* 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.,* 232: 313-321). Upon incubation of the purified apo-BlmI with [^{3}H-pantetheine]-CoA in the presence of the Sfp PPTase, we examined the covalent incorporation of the [^{3}H-pantetheine]-4'-phosphopantetheine moiety from CoA into holo-BlmI by autoradiographic analysis. Indeed, the apo-BlmI was quantitatively labeled by [^{3}H-pantetheine]-CoA, and no labeling was observed in the absence of either the apo-BlmI or the Sfp PPTase protein, demonstrating that the Sfp PPTase can recognize apo-BlmI as a substrate and specifically transfer the 4'-phosphopantetheine group from CoA into holo-BlmI.

In vitro aminoacylation of BlmI

Once we established BlmI as a type II PCP that can be readily modified by PCP-specific PPTases into the holo-BlmI protein, we tested if the holo-BlmI could be aminoacylated in trans, requiring an A domain. Since BlmI has no cognate A domain of its own, we turned our attention to another putative biosynthesis gene cluster we have cloned previously from *Sv* ATCC15003, which encodes at least four NRPS and one PKS modules. We have established that this gene cluster is not clustered with the *blm* locus and is unrelated to BLM biosynthesis. From this gene cluster, we amplified by PCR a 1579 bp fragment encoding an A domain, named Val-A, which we predicted to have a molecular weight of 56,581 and a pI of 7.39. We cloned *val-A* into pET-28a to yield pBS3, in which Val-A would be produced as a fusion protein with a His_6-tag at the N-terminus. Introduction of pBS3 into *E. coli* BL21(DE3) under the standard overexpression conditions recommended by the manufacturer (Novagen) resulted in good overproduction of Val-A, predominantly in soluble form, from which Val-A was purified by affinity chromatography using Ni-NTA resin. The purified Val-A protein was active by the amino acid-dependent ATP-PPi exchange assay (Lee and Lipmann (1970) *Method Emzymol.* 43: 585-602; Ku *et al.* (1997) *Chem. Biol.,* 4: 203-207). Among the 23 amino acids tested, Val-A specifically activated valine, an amino acid that is not required for BLM biosynthesis.

To carry out the aminoacylation in trans, we incubated the purified holo-BlmI and Val-A in vitro in the presence *L*-[^{14}C(U)]valine and ATP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). The aminoacylated holo-BlmI-*L*-[^{14}C(U)]valine species was subjected to SDS-PAGE and specific attachment of *L*-[^{14}C(U)]valine to holo-BlmI was determined by autoradiographic analysis. Remarkably, the holo-BlmI was specifically labeled by *L*-[^{14}C(U)]valine in the presence of Val-A, indicative of the formation of the holo-BlmI-S-valine thioester. The in trans aminoacylation between the holo-BlmI and Val-A proteins appeared to be very specific. Neither incubation of *L*-[^{14}C(U)]valine with Val-A, the apo-BlmI, or the holo-BlmI protein alone, nor incubation of *L*-[^{14}C(U)]valine with the Val-A and apo-BlmI proteins, resulted in the detection of ^{14}C-labeled BlmI protein.

Discussion.

Nonribosomal peptides and polyketides are two distinct classes of natural products yet are assembled from amino acids and short carboxylic acids by NRPSs and PKSs, respectively, in strikingly similar strategies (Cane *et al.* (1998) *Science* 282: 63-68).

These fascinating multifunctional enzyme complexes have been classified into two types based on their gene organization and enzyme architecture. Type I enzymes are multifunctional proteins consisting of domains for individual enzyme activities, and type II enzymes are multienzyme complexes consisting of discrete proteins that are largely monofunctional. While both type I and type II PKSs (Fig. 11A and 11C) have been well characterized to account for the vast structural diversities found in polyketide biosynthesis (Hopwood (1997) *Chem Rev.* 97: 2465-2497), all NRPSs studied so far are exclusively the type I modular enzymes (Fig. 11B) (Kleinkauf and von Döhren: H. (1996) *Eur. J. Biochem.* 236: 335-351; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675-2705). It is very tempting to speculate the existence of a type II NRPS that, analogous to type II PKS (Shen and Hutchinson (1993) *Science* 262: 1535-1540; Bao *et al.* (1998) *Biochemistry* 37: 8132-8138; Carreras and Khosla (1998) *Biochemistry* 37: 2084-2088), should consist of discrete proteins possessing enzyme activities such as the A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169), the PCP (Stein and Morris (1996) *J. Biol. Chem.* 271: 15428-15435), or the C (Stachlhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781) domains of type I NRPSs (Fig. 11D). The fact that both the A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169; Konz *et al.* (1997) *Chem. Biol.* 4: 927-937; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584; Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850) and the PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-15841; Pfeifer *et al.* (1995) *Biochemistry* 34: 7450-7459; Haese *et al.* (1994) *J. Mol. Biol.* 243: 116-122; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Gehring *et al.* (1996) *Chem. Biol.* 4: 17-24; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) domains of type I NRPSs can act as independent enzymes supports the hypothesis of a type II NRPS.

We have now cloned and sequenced the *blmI* gene, overproduced and characterized the BlmI protein as a bona fide type II PCP, and demonstrated that holo-BlmI can be aminoacylated by a completely unrelated A domain, providing for the first time genetic and biochemical evidence for a type II NRPS enzyme. We concluded BlmI as a type II PCP based on the following criteria. (1) The deduced amino acid sequence of the *blmI* gene is highly homologous to various PCP domains of known NRPSs, in particular at the signature motif of LGGXS within which the 4'-phosphopantetheine prosthetic group is covalently attached to the serine residue (Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; Stachelhaus and Marahiel (1995) *FEMS Microbiol. Lett.* 125: 3-14). While the current boundaries for a PCP domain in the literature were defined arbitrarily (Stachelhaus *et al.*

(1996) *Chem. Biol.* 3: 913-921) and varied from one PCP to another, we can now re-define a PCP domain for the type I NRPS as a 90 amino acid peptide with approximately 45 amino acids, each flanking the essential serine residue in the LGGXS (SEQ ID NO:81) motif, in light of this discrete BlmI type II PCP (Fig.9). (2) The *blmI* gene has been successfully expressed in *E. coli*, and fusion of a short peptide to the N-terminus of BlmI dramatically improved its overproduction efficiency. While we cannot exclude the effect of different systems on gene expression, i.e., *E. coli* M15(pREP4)(pBS1) vs. *E. coli* BL21(DE-3)(pBS2), we attribute the increase in expression efficiency to the stability of BlmI as an N-terminal fusion protein instead of the otherwise labile BlmI protein with its native N-terminus. Since BlmI was produced predominantly in the apo-form in *E. coli*, apo-BlmI apparently was not a substrate for the endogenous PPTases, such as EntD or ACP synthase, excluding BlmI as an ArCP or ACP, respectively. EntD and ACP synthase are known to 4'-phosphopantetheinylate apo-ArCP and ACP, respectively, to their holo-forms efficiently (Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Walsh *et al.* (1997) *Curr. Opin. Chem. Biol.* 1: 309-315; Lambalot and Walsh (1995) *J. Biol. Chem.* 270: 24658-24661). (3) The apo-BlmI protein serves as a substrate for PCP-specific PPTases that transfer the 4'-phosphopantetheine moiety from CoA to apo-BlmI to yield the holo-BlmI protein. We have demonstrated this posttranslational modification for BlmI in vivo with the Gsp PPTase (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) and *in vitro* with the Sfp PPTase (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595), both of which have been extensively used in preparing holo-PCPs. (4) The specific modification of apo-BlmI by 4'-phosphopantetheinylation has been monitored by HPLC analysis (Fig. 10) (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584) and by specific incorporation of [3-^{3}H]-β-alanine *in vivo* (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207; Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954) and of [^{3}H-pantetheine]-CoA *in vitro* (Gehring *et al.* (1998) *Biochemistry* 37: 11637-11650; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595), respectively, into the 4'-phosphopantetheine moiety of the holo-BlmI protein. The identity of BlmI was finally confirmed by MALDI-Tof mass spectral analysis that determined the molecular weight for both the apo- and holo-BlmI proteins.

While individual domains of type I NRPSs can function independently and several A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169; Konz *et al.*

(1997) *Chem. Biol.* 4: 927-937; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584; Mootz and Marahiel (1997) *J. Bacteriol.* 179: 6843-6850) and PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-15841; Pfeifer *et al.* (1995) *Biochemistry* 34: 7450-7459; Haese *et al.* (1994) *J. Mol. Biol.* 243: 116-122; Lambalot *et al.* (1996) *Chem. Biol.* 3: 923-936; Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Gehring *et al.* (1996) *Chem. Biol.* 4: 17-24; Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) domains have been overproduced, purified, and biochemically characterized, aminoacylation in trans has been successful only between PCPs and their cognate A domains (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). No aminoacylation between PCP and A domains from different NRPS modules has been observed. These results led to the conclusion that there is a specific protein-protein recognition between the A domain and its cognate PCP (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). Such domain-specific aminoacylation, in fact, should be beneficial in maintaining the fidelity of a type I NRPS by providing additional "gating" against misincorporation of non-specifically activated aminoacyl adenylate into the final peptide product. Since a type II PCP such as BlmI lacks its cognate A domain, we asked if BlmI could be aminoacylated by an unrelated A domain of a type I NRPS. Although we have yet to determine the biochemical role of BlmI in vivo, the fact that the *blmI* gene is located in the middle of the *blm* gene cluster suggests that it may be involved in BLM biosynthesis. To avoid the ambiguity of selecting an A domain that may potentially interact with BlmI in vivo, we preferred not to choose any A domain from the *blm* gene cluster to test if it could aminoacylate BlmI in trans. We reasoned that an A domain that is unrelated to BlmI should come from a gene cluster independent from BLM biosynthesis and should activate an amino acid not required by BLM. We chose Val-A because it satisfied both requirements. Val-A is an A domain of a type I NRPS from a gene cluster we have cloned previously from *Sv* ATCC15003 that has proven to be unrelated to BLM biosynthesis, and it specifically activates valine among the 23 amino acids tested. Remarkably, BlmI was efficiently aminoacylated by Val-A. The valine residue is specifically attached in a thioester linkage to the terminal -SH of the 4'-phosphopantetheine moiety of the holo-BlmI protein, as evidenced by the fact that the apo-BlmI was inactive under the identical conditions.

Aminoacylation of holo-BlmI by Val-A represents the first example in which an A domain aminoacylates a protein other than its cognate PCP domain. Since it has been suggested that an A domain of a type I NRPS can transfer the activated aminoacyl adenylate only to its cognate PCP domain because of the specific protein-protein recognition between the two domains (Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584), the fact that BlmI is aminoacylated by Val-A revealed a distinct feature of a type II PCP. It is very tempting to speculate that type II PCPs such as BlmI may have broad intrinsic substrate specificity toward either the aminoacyl adenylate, the A domain, or both. In fact, the latter feature is reminiscent of the type II PKS ACPs, which have been shown to be interchangeable among different PKS complexes (Shen and Hutchinson (1993) *Science* 262: 1535-1540; Bao *et al.* (1998) *Biochemistry* 37: 8132-8138; Carreras and Khosla (1998) *Biochemistry* 37: 2084-2088). The biosynthesis of *D*-alanyl-lipoteichoic acid in *Bacillus suntillis* (Perego *et al.* (1995) *J. Biol. Chem.* 270: 15598-15606) and *Lactobacillus casei* (Debabov *et al.* (1996) 178: 3869-3876) also involves a discrete ACP-like protein, the *D*-alanyl carrier protein, although the latter clearly is structurally and functionally different from PCPs.

The results strongly suggest the existence of a type II NRPS. In fact, we have already identified within the *blm* gene cluster two additional genes, *blmII* and *blmXI* (Fig. 1B), which encode type II C proteins based on sequence analysis (*see* Example 1).

Significance.

All NRPSs known to date are exclusively the type I modular enzymes that are multifunctional proteins consisting of domains, such as A (Stachlhaus and Marahiel (1995) *J. Biol. Chem.* 270: 6163-6169), PCP (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921), and C (Stachlhaus *et al.* (1998) *J. Biol. Chem.* 273: 22773-22781), for individual enzyme activities (Kleinkauf and von Döhren: H. (1996) *Eur. J. Biochem.* 236: 335-351; Marahiel *et al.* (1997) *Chem. Rev.* 97: 2651-2673; von Döhren *et al.* (1997) *Chem. Rev.* 97: 2675-2705), and control the structural variations of the resulting peptide products by the multiple-carrier thiotemplate mechanism (Cane *et al.* (1998) *Science* 282: 63-68; Stein and Morris (1996) *J. Biol. Chem.* 271: 15428-15435). While individual domains of type I NRPSs can function independently, aminoacylation in trans has been successful only between PCPs and their cognate A domains (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). We have cloned and sequenced the *blmI* gene, overproduced and characterized the BlmI protein as a bona fide type II PCP, and demonstrated that the holo-BlmI can be aminoacylated by a completely unrelated A domain. Our results provided for the first time the genetic and biochemical evidence to support the hypothesis of a type II NRPS, setting the stage for formulating new research concepts to study peptide biosynthesis. Genetic manipulation of type I NRPS has already been successful in generating novel peptides (Stachlhaus *et al.* (1995) *Science* 269: 69-72). An unprecedented type II NRPS should shed new light in engineering NRPS proteins, greatly increasing our ability to access peptides with even greater structural diversities.

Materials and methods

General DNA manipulations

Plasmids preparation and DNA extraction were carried out by using commercial kits (Qiagen, Santa Clarita, CA), and all other manipulations were carried out according to standard methods (Sambrook *et al.* (1989) *Molecular cloning: a laboratory manual:* (2nd ed): Cold Spring Harbor Laboratory Press: Cold Spring Harbor: USA). *E. coli* strain DH5α was used as the host for general DNA propagations.

Overexpression *of blmI in E. coli and purification of the BlmI protein*

The *blmI* gene was amplified from *Sv* ATCC15003 by PCR using a forward primer of 5'-CCG CCC ATG GGT GCT CCG CGT GGC GAG CGG ACC CGG CGC-3' (SEQ ID NO:82, the *Nco*I site is underlined) and a reverse primer of 3'-CCT AGA TCT CCG GTC CCG CTC CCC CGT-5' (SEQ ID NO:83, the *Bgl*II site is underlined). In order to create the *Nco*I site, the original starting sequence of "ATG AGC" has been changed to "ATG GGT", which resulted in the change of the second amino acid from serine to glycine. The first five codons of *blmI* were also optimized for overexpression in *E. coli*. The PCR-amplified 0.3 kb *Nco*I-*Bgl*II fragment was cloned into the similar sites of pQE-60 (Qiagen) to form pBS1. Digestion of pBS1 with *Nco*I and *Hind*III and cloning the resulting 0.3 kb *Nco*I-*Hind*III fragment into the same sites of pET-29a (Novagen, Madison, WI) yielded pBS2.

Expressions of *blmI* in *E. coli* M15 (pREP4)(pBS1) and in *E. coli* BL-21(DE-3)(pBS2) and purification of the resulting BlmI protein by affinity chromatography on Ni-NTA resin were carried out under the standard conditions recommended by Qiagen and Novagen, respectively. The incubation temperature was lowered to 30 °C to improve the solubility. The purification of BlmI was monitored by SDS-PAGE on 15% gel. The final pure BlmI protein was desalted on PD-10 column (Sephadex G-25, Pharmacia Biotech, Piscataway, NJ) into 50 mM sodium phosphate buffer, pH 7.8, containing 200 mM NaCl, 10 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 1 mM EDTA, 10% glycerol, and stored at - 80 °C for in vitro assays.

HPLC analysis and MALDI-Tof mass spectral determination

Samples of BlmI (30-70 μg) purified from *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp) were analyzed on a Nova-Pak C18 column (5mm x 10, Waters, Milford, MA) using a Rainin DMAX HPLC unit. The column was developed by a linear gradient of 0-50% acetonitrile in 0.1% trifluoroacetic acid in 25 min, followed by additional 5 min at 50 % acetonitrile, with a flow rate of 0.6 ml/min and detection at 280 nm. MALDI-Tof mass spectral determination was performed on a Bruker Biflex IIII spectrometer at the Facility for Advanced Instrumentation of University of California, Davis.

In vivo labeling of BlmI with [3-^{3}H]-β-alanine

The β-alanine auxotroph *E. coli* strain OG7001 (Epple *et al.* (1998) *J. Bacteriol.* 180: 4950-4954) was transformed with pBS2 and cultured under the same conditions as for *E. coli* BL21(DE3) (Novagen). For co-expression of *blmI* with *gsp*, pDPT-Gsp (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207) was similarly transformed into *E. coli* OG7001(pBS2) and the transformants were cultured in 2xYT (Debabov *et al.* (1996) 178: 3869-3876) in the presence of kanamycin (25 μg/ml) and chloramphenicol (50 μg/ml). For in vivo labeling experiment, cells from 2 ml overnight culture of either *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp) were harvested, washed with M9 minimal medium (Debabov *et al.* (1996) 178: 3869-3876), and re-suspended in 2 ml of M9 minimal medium. The latter were used as seed cultures (20 μl) to inoculate 1 ml M9 medium with kanamycin (25 μg/ml) or kanamycin (25 μg/ml) and chloramphenicol (50 μg/ml) for *E. coli* OG7001(pBS2) or *E. coli* OG7001(pBS2/pDPT-Gsp), respectively. The resulting culture was incubated at 30 °C, 250 rpm to OD_{600nm} 0.6 and to this was added 10 μCi of [3-^{3}H]-β-alanine (50 Ci/mmol, American Radiolabeled Chmicals Inc., St. Louis, MO) with or without IPTG (1 mM). Total proteins were resolved by SDS-PAGE on 15% gels that were Coomassie blue-stained. To determine ^{3}H-labeling of the overproduced holo-BlmI protein, gels were soaked in Amplifier (Amersham, Arlington Heights, Il) for 20 min, dried between two sheets of cellulose membrane (KOH Development Inc., Ann Arbor, MI), and visualized by autoradiography on X-ray films (Fuji Medical Systems, Stamford, CT).

In vitro labeling of BlmI with [^{3}H-pantetheine]-CoA

Expression of *sfp* in *E. coli* MV1190(pUC8-Sfp), purification of the Sfp PPTase to homogeneity, and 4'-phosphopantetheinylation of apo-BlmI by Sfp in vitro were carried out essentially according to literature procedures (Quadri *et al.* (1998) *Biochemistry* 37: 1585-1595; Nakano *et al.* (1992) *Mol. Gen. Genet.* 232: 313-321). A typical 100 μl assay solution contained 26 μM apo-BlmI, 2.9 μM Sfp, 25 μM [^{3}H-pantetheine]-CoA (0.9 μCi, 40 Ci/mM), 10 mM MgCl$_2$, and 5 mM DTT, in 75 mM MES/NaOAc buffer, pH 6.0. After 30 min incubation at 37 °C, the assays were stopped by addition of 5 μl of bovine serum albumin (0.2 mg/ml) and 0.9 ml of cold 10% (v/v) trichloroacetic acid (TCA). The precipitated proteins were collected by centrifugation at 14,000 rpm, 20 min, 4 °C (Eppendorf 5415C centrifuge), washed with 10% TCA three times, and resolved by SDS-PAGE on 15% gel. The ^{3}H-activity incorporated into holo-BlmI was similarly determined by autoradiography as described for in vivo labeling of holo-Blm with [3-^{3}H]-β-alanine.

Overexpression of val-A in E. coli and purification and assay of the Val-A protein

The *val-A* fragment was amplified from *Sv* ATCC15003 by PCR using a forward primer of 5'-GGA ATT CCA TAT GGG CAC CAC CGT CGC CGC G-3' (SEQ ID NO:84, the *Nde*I site is underlined), and a reverse primer of 3'-GGC AAG CTT GGG ACC GGG CGT GGA GCG C (SEQ ID NO:85, the *Hind*III site is underlined). The PCR-amplified 1.6 kb *Nde*I-*Hind*III fragment was cloned in the similar sites of pET-28a (Qiagen) to yield pBS3. Expression of *val-A* in *E. coli* BL-21(DE-3)(pBS3) and purification of the resulting Val-A protein by affinity chromatography on Ni-NTA resin were carried out under the standard conditions recommended by Novagen.

Amino acid-dependent ATP-PPi assays were performed essentially according to the literature procedures (Ku *et al.* (1997) *Chem. Biol.* 4: 203-207; Lee and Lipmann (1970) *Method Emzymol.* 43: 585-602). A typical 100 μl assay solution contained 180 nM Val-A, 1 mM ATP, 0.1 mM PPi with 0.2 μCi of ^{32}P-PPi (11.75 Ci/mmol, NEN Life Science Products, Inc., Boston, MA), 1 mM MgCl$_2$, 0.1 mM EDTA, and 1 mM *L*-amino acid in 50 mM sodium phosphate buffer, pH 7.8. After 30 min incubation at 30°C, the assays were stopped by addition of 0.9 ml of cold 1% (w/v) activated charcoal in 3% (v/v) perchloric acid. The precipitates were collected on glass fiber filters (2.4 cm, G-4, Fisher, Pittsburgh, PA), washed successively with 10 ml of 0.2 M sodium phosphate buffer, pH 8.0, 4 ml water, and 1 ml of ethanol, and dried in air. The filters were mixed with 7 ml of scintillation fluid (ScintiSafe Gel, Fisher) and counted on a Beckman LS-6800 scintillation counter to determine the radioactivity.

In vitro aminoacylation of holo-BlmI by Val-A

The aminoacylation of holo-BlmI was carried out essentially according to literature methods (Stachelhaus *et al.* (1996) *Chem. Biol.* 3: 913-921; Weinreb *et al.* (1998) *Biochemistry* 37: 1575-1584). A typical 100 μl assay solution contained 180 nM Val-A, 1.5-2.8 μM apo- or holo-BlmI, 35 μM *L*-[^{14}C(U)]-valine (283 mCi/mmol, NEN Life Science Products, Inc., Boston, MA), 5 mM ATP, 10 mM $MgCl_2$, and 5 mM DTT in 75 mM Tris-HCl buffer, pH 8.0. The reactions were started by the addition of ATP and, after incubation at 37 °C for 30 min, were stopped by addition of 0.9 ml of cold 7% (v/v) TCA. The precipitated proteins were collected by centrifugation at 14,000 rpm, 20 min, 4 °C (Eppendorf 5415C centrifuge) and resolved by SDS-PAGE on a 15% gel. The radioactivity incorporated into the holo-BlmI-*L*-[^{14}C(U)]valine species was similarly determined by autoradiography as described for in vivo labeling of holo-BlmI with [3-^{3}H]-β-alanine.

Example 3:

Cloning and characterization of a phosphopantetheinyl transferase from the bleomycin-producing *Streptomyces verticillus* ATCC15003

Multienzymes complexes exist for acyl group activation and transfer reactions in the biogenesis of fatty acids, the polyketide family of natural products (*e.g.* erythromycin, tetracycline), and almost all non-ribosomal peptides (*e.g.* vancomycin, cyclosporin, penicillin). All of these complexes contain one or more small proteins, ~80-100 amino acids long, either as separate subunits or as integrated domains, that function as carrier proteins for the growing acyl chain (acyl-, peptidyl-, and aryl- carrier proteins, abbreviated as ACP, PCP, and ArCP). They are converted from inactive apo-forms to functional holo-forms by the covalent attachment of the 4'-phosphopantetheine moiety of coenzyme A to a conserved serine residue of the carrier-protein substrate. This essential post-translational modification is catalyzed by a family of enzymes known as phosphopantetheinyl transferases (PPTases) (Lambalot et al. *Chem. Biol.* (1996) 3:923-936; Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315).

Research in the field of polyketide and non-ribosomal peptide biosynthesis has been hampered by the inability to fully modify and thus convert to the active form some polyketide synthases (PKS) and polypeptide synthetases (NRPS) when overproduced in heterologous hosts, presumably because the host PPTases are unable to effectively modify these overexpressed protein substrates. Our group is currently involved in the characterization of the gene cluster responsible for the biosynthesis of the antitumor drug bleomycin in *Streptomyces verticillus* ATCC15003. As bleomycin synthetase is a hybrid NRPS/PKS enzyme, we decided to obtain a PPTase from the producing organism in order to use it *in vitro* or *in vivo* by coexpression with the synthetase genes to produce properly modified, active synthetases for our studies.

Results and Discussion

Cloning of the *pttA* gene from *S. verticillus* ATCC15003.

The similarities among PPTases from different organisms are reduced to two short motifs separated by 40-45 residues: (V/I)G(V/I)D, and (F/W)(S/C/T)XKE(A/S)hhK (Lambalot et al. *Chem. Biol.* (1996) 3:923-936; Walsh etal. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Our previous attempts to amplify PPTase sequences from *S. verticillus* chromosomal DNA using degenerate primers according to the two conserved motifs were unsuccessful (unpublished results), so we decided to narrow our target. PPTases have been classified in two groups, according to their specificity for the carrier-protein substrate: PPTases involved in polyketide/fatty acid biosynthesis use acyl carrier proteins (ACPs) as substrate, while those for non-ribosomal peptide biosynthesis use peptidyl carrier proteins (PCPs) or aryl carrier proteins (ArCPs) (Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Several "NRPS-type" PPTase sequences were used to screen the databases to look for actinomycete homologues, and four proteins of unknown function were found: NshC from *Streptomyces actuosus* (Li et al. *Gene* (1990) 91:9-17), SC5A7. 23 from *S. coelicolor* (GenBank AL031107), an unnamed protein from *Streptomyces* sp. strain TH1 (Mori et al. *J. Bacteriol.* (1997) 179:5677-5683), and Rv2794c (later renamed PptT (Quadri et al. *Chem. Biol.* (1998) 5:631-645)) from *Mycobacterium tuberculosis* (GenBank AL008967). The alignment of the actinomycete sequences showed the two motifs conserved in all PPTases and an additional motif - the "THC" motif: PXWPXGX$_2$GS(M/L)THCXGY (SEQ ID NO:86), located about 15 amino acids upstream of the (V/I)G(V/I)D motif (SEQ ID NO:87). The "THC" motif is not universally conserved in all PPTases, but it can be detected also in some non-actinomycete PPTases like EntD (Coderre et al. *J. Gen. Microbiol.* (1989) 135:3043-3055). Using a recently developed method of PCR primer design (the CODEHOP strategy (COnsensus-DEgenerate Hybrid Oligonucleotide Primer) (Rose et al. *Nucleic Acids Res.* (1998) 26:1628-1635), two primers were designed around the typical C-terminal PPTase motif (primers KEA-1: 5'-T GCA GCA GAA CAG GAG GCK NYC CCA

NKG-3' (SEQ ID NO:88) and KEA-2: 5'-TG GGT CAG CGG GTA CCA NRC YTT RWA-3' (SEQ ID NO: 89, H=C+A, N=A+C+T+G, Y=C+T, K=G+T, R=A+G, W=T+A)), and one primer was designed from the "THC" motif (primer THC: 5'-C GGC ATG GTC GGC TCC HTN ACN CAY TG-3', SEQ ID NO:90, H=C+A, N=A+C+T+G, Y=C+T, K=G+T, R=A+G, W=T+A); this motif is not universally conserved in PPTases of all organisms). Using *S. verticillus* chromosomal DNA as template, no amplification product was detected using the THC and the KEA-1 primers. The set of primers THC/KEA-2 successfully amplified a single band of the expected size (about 250 bp), which was gel-purified and cloned. Eight individual clones were sequenced, and all of them resulted to be identical (except differences due to primer utilization) and highly similar to the putative actinomycete PPTases. The PCR fragment was used as a probe to screen a *S. verticillus* genomic library by colony hybridization. Of the 10,000 colonies screened, 25 positive clones were identified, and then confirmed by Southern analysis to contain the same 4. 6-kb *Bam*HI hybridizing band. The 4. 6-kb DNA fragment was subcloned, and the nucleotide sequence of a 1,761-bp *Bam*HI-*Sal*I region was determined (SEQ ID NO. 3).

Sequence analysis of the *pptA* locus.

The sequence of the 1,761-bp *Bam*HI-*Sal*I fragment was analyzed for coding regions by using the CODONPREFERENCE and TESTCODE programs of the GCG package (Genetics Computer Group, Madison, Wisconsin). Two complete ORFs (*pptA, orf3*) and two incomplete ORFs (*orf1, orf4*) were identified within the sequenced region (Figure 13). The first ORF from left to right (designated *orf1*) starts out of the analyzed area and ends with a TGA codon at position 248 of the sequenced fragment. Comparison of the deduced product of *orf1* with proteins in databases showed similarities with Rv2795c from *Mycobacterium tuberculosis* (GenBank AL008967) and SC5A7. 22 from *S. coelicolor* (GenBank AL031107), both of unknown function. The second ORF, *pptA*, contains the sequence amplified by PCR and used for the cloning of this locus. It comprises 741 nucleotides, starting with a GTG codon (position 245) which is coupled to the stop codon of *orf1*, and ending with a TAA codon. The starting codon of *pptA* is preceded by a potential ribosomal binding site (RBS), GGGAG. The overall (76. 6%) and third codon position (93. 9%) G+C contents and the codon usage of *pptA* are similar to those found in other *Streptomyces* genes, with the exception of the stop codon (TAA), which is most uncommon in this group of organisms (Wright et al. *Gene* (1992) 113:55-65). The *pptA* gene encodes a protein of 246 amino acids with a predicted molecular mass of 25,619 Da and a pI of 4. 76, which contains the conserved PPTase motifs. Databases searches with PptA showed significant similarities to the putative actinomycete PPTases (39-52%/48-61% identity/similarity) and to confirmed bacterial PPTases such as EntD from *E. coli* (17%/24% identity/similarity) (Lambalot et al. *Chem. Biol.* (1996) 3:923-936). The third ORF, *orf3*, is separated from *pptA* by an apparently noncoding DNA region of 153 bp, and it is transcribed in opposite and convergent direction with respect to *orf1-pptA*. The gene *orf3* comprises 240 nucleotides, starting with an ATG codon (position 1358) and ending with TGA. The starting codon of *orf3* is preceded by the sequence GAAGG, a potential RBS. The deduced product of *orf3* encodes a protein of 79 amino acids with a predicted mass of 7,555 Da and a pI of 7. 17. The Orf3 protein shows similarities to the N-terminal region of SC5H1. 35c, a protein of unknown function from *S. coelicolor* (GenBank AL049863). Analysis of Orf3 with the SignalP program (Nielsen et al. *Protein Engineer.* (1997) 10:1-6) predicts an N-terminal signal peptide which would be cleaved between residues 27 and 28 (ALA-DS), suggesting that the mature protein (52 amino acids, 5,099 Da, pI 4. 31) would be secreted. Between *orf3* and *orf4* there is an apparently noncoding region of 251 nucleotides. The *orf4* gene is transcribed in opposite and divergent direction with respect to *orf3*. It starts with an ATG codon at position 1610, preceded by a potential RBS (GGAGG), and ends out of the sequenced fragment. The deduced protein product (50 amino acids) of the incomplete *orf4* contains a potential NAD/FAD binding motif, $GXGX_2GX_3GX_6G$ (Scrutton et al. *Nature* (1990) 343:38-43), showing low similarities to diverse oxidoreductases.

Heterologous expression and biochemical characterization of PptA.

In order to test if *pptA* actually encodes a functional PPTase, we decided to overproduce and purify the PptA protein, and assay its catalytic competence on putative substrate proteins or domains. The *pptA* coding sequence was amplified by PCR and cloned into the T5-promoter-based pQE-70 vector, yielding plasmid pQEPPT, in such a way that a hexahistidine tag would be added at the C-terminus of the protein. Expression of the pQEPPT construct in *E. coli* M15(pREP4) resulted in the overproduction of soluble His-tagged PptA which was readily purified by affinity chromatography on Ni-NTA agarose under non-denaturing conditions (FIGURE). Because *pptA* belongs, by sequence similarity, to the subfamily of PPTases involved in nonribosomal peptide synthesis, we first assayed its activity using two different apo-PCPs as protein substrates. The first one, BlmI, has been previously characterized in our laboratory as a discrete peptidyl carrier protein, or type II PCP, whose gene is found within the bleomycin-biosynthesis gene cluster of *S. verticillus*

(Du et al. *Chem. Biol.* (1999) 6:507-517). For the second PCP substrate we used BlmX, a bimodular NRPS protein encoded in the same cluster (Fig. 2), as a source of a type I PCP, i. e. a PCP included in a multidomain NRPS. For the production of this type I PCP, we amplified by PCR a 1,898 bp fragment encoding the adenylation and PCP domains from the second module of BlmX. This DNA fragment was cloned into pMAL-c2x to yield pMAL1617, in which the type I PCP would be produced as a maltose-binding protein (MBP) fusion, MBlmX-2, with a predicted molecular mass of 108. 5 kDa. Introduction of pMAL1617 in *E. coli* TB1 resulted in good overproduction of MBlmX-2, about 40% soluble, which was purified by affinity chromatography using amylose resin. To test the PPTase activity, we incubated the purified PptA with BlmI and MBlmX-2 as putative protein substrates in the presence of (^{3}H)-(pantetheinyl)-CoASH, and the tritiated products were subjected to SDS electrophoresis and autoradiography. The well-characterized PPTase Sfp from *B. subtilis*, which exhibits a broad specificity for its protein substrate (Quadri et al. *Biochemistry* (1998) 37:1585-1595), was included as a positive control. In these experiments PptA exhibited a robust phosphopantetheinylation activity on both BlmI and MBlmX-2. Having demonstrated that PptA does in fact have PPTase activity on both type I and type II PCP substrates from nonribosomal peptide synthetases, we then proceeded to test two different acyl-carrier proteins (ACPs) as potential substrates. The first one, BlmVIII, is a monomodular multidomain polyketide synthase (PKS) which is encoded in the bleomycin-biosynthesis gene cluster of *S. verticillus* (Fig. 2). BlmVIII contains an ACP domain at its C-terminus, that is a type I ACP. For the second ACP substrate we used TcmM, a type II acyl carrier protein involved in the biosynthesis of the aromatic polyketide tetracenomycin C in *S. glaucescens* (Shen et al. *J. Bacteriol.* (1992) 174:3818-3821; Bao et al. *Biochemistry* (1998) 37: 8132-8138). For the production of TcmM, its coding sequence was transferred from a construct previously made in pET-22b (Gehring et al. *Chem. Biol.* (1997) 4:17-24) into the pET-28a vector to yield pET28a-TcmM, in such a way that a hexahistidine tag should be added at both the N-terminus and the C-terminus of the protein. Plasmid pET28a-TcmM was introduced into *E. coli* BL21(DE3), and TcmM was easily purified by affinity chromatography using Ni-NTA resin. In vitro phosphopantetheinylation assays were performed as before, but using BlmVIII and TcmM as protein substrates, and PptA was able to posttranslationally modified both ACP substrates.

The *pptA* gene is not clustered to the bleomycin-biosynthesis locus.

Some bacterial PPTase genes have been found clustered, or close, to their respective "partner" NRPS genes: *entD* {enterobactin (Coderre et al. *J. Gen. Microbiol.* (1989) 135:3043-3055)}, *sfp* {surfactin (Cosmina et al. *Mol. Microbiol.* (1993) 8:821-831)}, *gsp* {gramicidin (Borchert et al. *J. Bacteriol.* (1994) 176:2458-2462)}, *bli* {bacitracin (Gaidenko et al. *Biotechnologia* (1992) 13-19)}, *lpa-14* {iturin (Huang et al. *J. Ferment. Bioeng.* (1993) 76:445-450)}. To test the possible clustering of *pptA* to the bleomycin-biosynthesis (*blm*) locus, PCR reactions were performed using the THC/KEA-2 primers on several overlapping cosmid clones spanning the *blm* locus plus 30-40 kb upstream and downstream of its putative limits. No amplification product could be obtained in these reactions, showing that the *pptA* gene is not clustered with the *blm* locus.

Discussion

It has been suggested that in organisms containing multiple phosphopantetheine-requiring pathways, each pathway has its own posttranslational modifying activity (Walsh et al. *Curr. Opin. Chem. Biol.* (1997) 1:309-315). Our group has found that *S. verticillus* ATCC15003 contains several PKS and NRPS gene clusters, one of them being responsible for bleomycin production (a hybrid NRPS/PKS system) (Shen et al. *Bioorg. Chem.* (1999) 27:155-171; Du et al. *Chem. Biol.* (1999) 6:507-517). This suggested that the gene encoding the PPTase for the BLM NRPS could be also clustered, or close, to the NRPS genes. However, we have not found this gene after sequencing almost the whole *blm* NRPS locus. Because having this gene could be important for us in order to express functional NRPS modules from the *blm* cluster, we decided to clone the PPTase gene. Additionally, if the "one NRPS cluster - one PPTase" hypothesis was true, it seemed possible to use PPTase sequences as a new kind of probe to clone novel NRPS clusters.

We know that in *S. verticillus* there are several NRPS locus (maybe four), so we expected several "PCP-type" PPTases. However we have amplified only one, and it does not seem to be closely linked to any of the NRPS loci. Interestingly in the actinomycete *Mycobacterium tuberculosis*, whose genome is fully sequenced, there is only one PCP-type PPTase gene, which is not clustered with any of the two NRPS loci present in this organism (Quadri et al, *Chem. Biol.* (1998) 5:631-645). These and other indirect evidences suggest that the idea of cluster-specific PPTases is not the general rule at all but most probably the exception, especially in organisms containing multiple NRPS clusters. And there are strong evidences that at least some PCP-type PPTases can posttranslationally modify PCPs from different clusters and even different organisms (Quadri et al, *Chem. Biol.* (1998) 5:631-645; Gehring et al, *Biochemistry* (1998) 37:11637-11650). It is most likely that there is only one PCP-type PPTase in *S. verticillus* and that its gene is not necessarily clustered to any of the NRPS loci.

Biochemical characterization of the purified PptA protein confirmed not only its PPTase activity but also its broad specificity, comparable to that of Sfp. Different apo-PCPs (type I and type II) and a type-I apo-ACP from the bleomycin synthetase, and the type-II apo-ACP from the tetracenomycin PKS of *Streptomyces glaucescens* were efficiently used as substrates by PptA. These results suggest PptA as a good candidate for heterologous coexpression with NRPS and PKS genes to overproduce active holo-synthase enzymes.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of

a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41;

a nucleic acid encoding a polypeptide encoded by any one of *Blm* open reading frames (ORFs) 8 through 41; and

a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II and the nucleic acid of a bleomycin-producing organism as a template.

2. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

3. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

4. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

5. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI, blmII,* and *blmXI.*

6. An isolated nucleic acid comprising a nucleic acid encoding a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain,

an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

7. The isolated nucleic acid of claim 6, wherein said nucleic acid comprises a nucleic acid encoding one or more proteins comprising a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

8. The isolated nucleic acid of claim 7, wherein said nucleic acid comprises an open reading frame from SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 3.

9. An isolated nucleic acid comprising a nucleic acid encoding a protein encoded by a gene from a BLM gene cluster.

10. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmI, blmII, and blmXI.*

11. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

12. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by *blmVIII.*

13. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmI, blmII, and blmXI.*

14. The nucleic acid of claim 9, wherein said nucleic acid comprises a nucleic acid selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

15. The nucleic acid of claim 9, wherein said nucleic acid comprises *blmVIII.*

16. An isolated nucleic acid comprising a nucleic acid that encodes a protein comprising at least one catalytic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP)

domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain, and that hybridizes to a nucleic acid selected from the group consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40,* and *orf41* under stringent conditions.

17. The nucleic acid of claim 16, wherein said isolated nucleic acid comprises a nucleic acid encoding a module.

18. The nucleic acid of claim 16, wherein said isolated nucleic acid comprises a nucleic acid encoding a BLM gene.

19. An isolated nucleic acid comprising a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40,* and *orf41*, or an allelic variant thereof.

20. The nucleic acid of claim 19, wherein said nucleic acid comprises a nucleic acid that is a single nucleotide polymorphism (SNP) of a nucleic acid selected from the group consisting of consisting of *orf8*, *orf9*, *orf10*, *orf11*, *orf12*, *orf13*, *orf14*, *orf15*, *orf15*, *orf16*, *orf17*, *orf18*, *orf19*, *orf20*, *orf21*, *orf22*, *orf23*, *orf24*, *orf25*, *orf26*, *orf27*, *orf28*, *orf29*, *orf30*, *orf31*, *orf32*, *orf33*, *orf34*, *orf35*, *orf36*, *orf37*, *orf38*, *orf39*, *orf40,* and *orf41.*

21. An isolated gene cluster comprising open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin.

22. An isolated multi-functional protein complex comprising both a polyketide synthase (PKS) and a peptide synthetase (NRPS).

23. An isolated nucleic acid encoding a multi-functional protein complex comprising both a polyketide synthase (PKS) and a peptide synthetase (NRPS).

24. An isolated polypeptide comprising a catalytic domain encoded by a nucleic acid of a bleomycin gene cluster wherein said nucleic acid comprises a nucleic acid selected from the group consisting of

a nucleic acid encoding any one of *Blm* open reading frames (ORFs) 8 through 41; and

a nucleic acid amplified by polymerase chain reaction (PCR) using any one of the primer pairs identified in Table II.

25. The polypeptide ofclaim 25, wherein said polypeptide comprises an enzymatic domain selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

26. The polypeptide claim 25, wherein the nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least two open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

27. The polypeptide claim 25, wherein said nucleic acid of a bleomycin gene cluster comprises a nucleic acid encoding at least three open reading frames selected from the group consisting of *Blm* open reading frames 8 through 41.

28. The polypeptide claim 25, wherein said polypeptide comprises a C domain lacking one or more His residues of the conserved HHxxxDG active site for transpeptidation.

29. The polypeptide claim 25, wherein said polypeptide is a polypeptide encoded by a gene selected from the group consisting of *blmI, blmII,* and *blmXI.*

30. An isolated polypeptide comprising a module comprising two or more catalytic domains of a protein encoded by a nucleic acid of a bleomycin gene cluster wherein said catalytic domains are selected from the group consisting of a condensation (C) domain, an adenylation (A) domain, a peptidyl carrier protein (PCP) domain, a condensation/cyclization domain (Cy), an acyl-carrier protein (ACP)-like domain, an

oxidization domain (Ox), a ketoacyl synthase (KS) domain , an acetyl transferase (AT) domain, a ketoreductase (KR) domain, and a methyltransferase (MT) domain.

31. The polypeptide of claim 30, wherein said polypeptide comprises a module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

32. An isolated polypeptide encoded by a gene from a BLM gene cluster.

33. The polypeptide of claim 32, wherein polypeptide is encoded by a gene selected from the group consisting of *blmI, blmII, and blmXI.*

34. The polypeptide of claim 32, wherein said nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of *blmIII, blmIV, blmV, blmVI, blmVII, blmIX, and blmX.*

35. The polypeptide of claim 32, wherein polypeptide is encoded by *blmVIII.*

36. An isolated polypeptide comprising a module wherein said module is specifically bound by an antibody that specifically binds to a BLM module selected from the group consisting of NRPS-0, NRPS-1, NRPS-2, NRPS-3, NRPS-4, NRPS-5, NRPS-6, NRPS-7, NRPS-7, NRPS-9, and PKS.

37. The polypeptide of claim 36, wherein said polypeptide is specifically bound by an antibody that specifically binds to a polypepide encoded by a gene selected from the group consisting of of *blmI, blmII, blmXI, blmIII, blmIV, blmV, blmVI, blmVII, blmIX, blmX,* and *blmVIII.*

38. An isolated polypeptide comprising a polypeptide encoded an open reading frame of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, or an allelic variant thereof.

39. The polypeptide of claim 38, wherein said nucleic acid comprises a single nucleotide polymorphism (SNP) of an open reading of a nucleic acid selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

40. An expression vector comprising a nucleic acid of any one of claims 1 through 23.

41. A host cell transformed with an expression vector of claim 40.

42. The host cell of claim 41, wherein said cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

43. The cell of claim 41, wherein said cell is a bacterial cell.

44. The cell of claim 43, wherein said cell is a *Streptomyces* cell.

45. The cell of claim 41, wherein said cell is a eukaryotic cell.

46. A method of chemically modifying a biological molecule, said method comprising contacting a biological molecule that is a substrate for a polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames with the polypeptide encoded by one or more bleomycin biosynthesis gene cluster open reading frames, whereby said polypeptide chemically modifies said biological molecule.

47. The method of claim 46, wherein said method comprising contacting said biological molecule with at least two different polypeptides encoded by *blm* gene cluster open reading frames.

48. The method of claim 46, wherein said method comprising contacting said biological molecule with at least three different polypeptides encoded by *blm* gene cluster open reading frames.

49. The method of claim 46, wherein said contacting is in a host cell.

50. The method of claim 49, wherein said host cell is a bacterium.

51. The method of claim 46, wherein said contacting *ex vivo*.

52. The method of claim 46, wherein said biological molecule is an endogenous metabolite produced by said host cell.

53. The method of claim 46, wherein said biological molecule is an exogenous supplied metabolite.

54. The method of claim 46, wherein said host cell is a eukaryotic cell.

55. The method of claim 54, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, a yeast cell, a plant cell, a fungal cell, and an insect cell.

56. The method of claim 46, wherein said biological molecule is an amino acid and said polypeptide is a peptide synthetase.

57. The method of claim 46, wherein said polypeptide is a methyl transferase.

58. A method of coupling a first amino acid to a second amino acid, said method comprising contacting the first and second amino acid with a recombinantly expressed bleomycin nonribosomal peptide synthetase (NRPS).

59. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-5, NRPS-4, NRPS-3, NRPS-9, NRPS-8, and NRPS-7.

60. The method of claim 64, wherein said NRPS is selected from the group consisting of NRPS-6, NRPS-2, NRPS-1, and NRPS-0.

61. The method of claim 64, wherein said contacting is in a host cell.

62. A method of coupling a first fatty acid to a second fatty acid, said method comprising contacting the first and second fatty acids with a recombinantly expressed bleomycin polyketide synthase (PKS).

63. The method of claim 62, said contacting is in a host cell.

64. A method of producing a bleomycin or bleomycin analog, said method comprising:

providing a cell transformed with an exogenous nucleic acid comprising a bleomycin gene cluster encoding polypeptides sufficient to direct the assembly of said bleomycin or bleomycin analog;

culturing the cell under conditions permitting the biosynthesis of bleomycin or bleomycin analog; and

isolating said bleomycin or bleomycin analog from said cell.

65. An isolated nucleic acid comprising a nucleic acid encoding a phosphopantetheinyl transferase said nucleic acid encoding a phosphopantetheinyl transferase being selected from the group consisting of:

a nucleic acid encoding the protein encoded by the nucleic acid of SEQ ID NO:3;

a nucleic acid amplified by polymerase chain reaction (PCR) using primers that specifically amplify ORF 41 (primers: SEQ ID NO:71 and SEQ ID NO:72) and *Streptomyces* nucleic acid as a template;

a nucleic acid encoding a polypeptide having phosphopantetheinyl transferase activity where said nucleic acid specifically hybridizes to the nucleic acid of SEQ ID NO: 3 under stringent conditions.

66. The nucleic acid of claim 65, said nucleic acid comprising a nucleic acid of SEQ ID NO:3.

67. A polypeptide comprising a phosphopantetheinyl transferase encoded by SEQ ID NO:3.

68. A vector comprising the nucleic acid of claim 66.

69. A cell transfected with the vector of claim 68.

70. A method of converting an apo-carrier protein to a holo-carrier protein comprising reacting said apo-carrier protein with a recombinant phosphopantetheinyl transferase encoded by SEQ ID NO:3 and coenzyme A thereby producing a holo-carrier protein.

71. A cell comprising a modified bleomycin gene cluster nucleic acid, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

72. The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin bene cluster.

73. The cell of claim 72, wherein said resistance gene is a gene listed in Table III.

BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES

ABSTRACT OF THE DISCLOSURE

This invention provides detailed sequence analysis and characterization of the gene cluster responsible for the synthesis of bleomycin in *Streptomyces verticillus*. The bleomycin gene cluster provides the first hybrid polyketide synthase/nonribosomal peptide synthetase pathway and elucidation of the various modules and enzymatic domains characterizing the pathway provides convenient synthetic routes for bleomycins, bleomycin analogs, and various other polyketides.

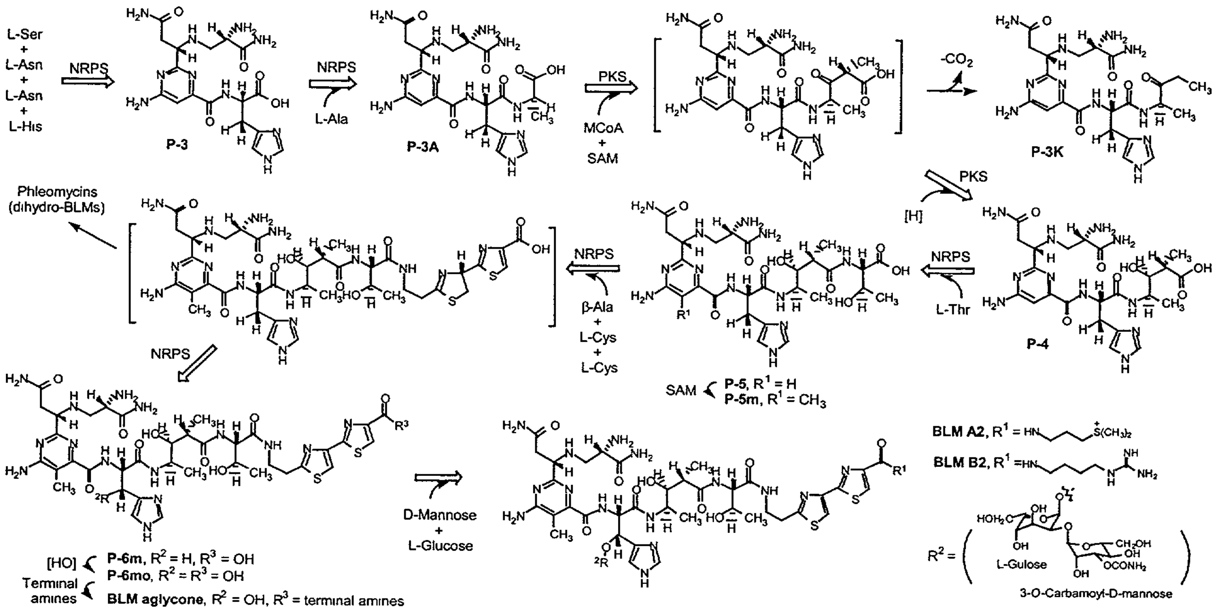

L-Ser + L-Asn + L-Asn + L-His
NRPS
P-3
NRPS
L-Ala
P-3A
PKS
MCoA + SAM
-CO2
P-3K
PKS
[H]
P-4
NRPS
L-Thr
SAM
P-5, R1 = H
P-5m, R1 = CH3
NRPS
β-Ala + L-Cys + L-Cys
Phleomycins (dihydro-BLMs)
NRPS
[HO]
P-6m, R2 = H, R3 = OH
P-6mo, R2 = R3 = OH
Terminal amines
BLM aglycone, R2 = OH, R3 = terminal amines
D-Mannose + L-Glucose
BLM A2, R1 =
BLM B2, R1 =
R2 =
L-Gulose
3-O-Carbamoyl-D-mannose

Fig. 1A

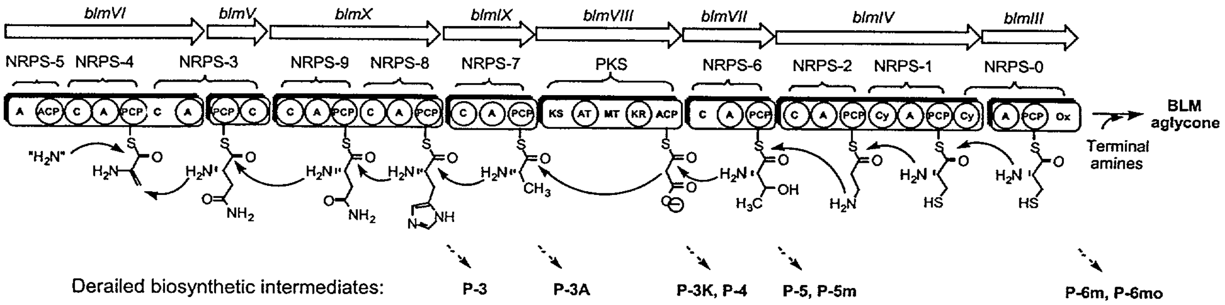

blmVI
blmV
blmX
blmIX
blmVIII
blmVII
blmIV
blmIII
NRPS-5 NRPS-4
NRPS-3
NRPS-9
NRPS-8
NRPS-7
PKS
NRPS-6
NRPS-2
NRPS-1
NRPS-0
A ACP C A PCP C A
PCP C
C A PCP C A PCP
C A PCP
KS AT MT KR ACP
C A PCP
C A PCP Cy A PCP Cy
A PCP Ox
BLM aglycone
Terminal amines
"H2N"
Derailed biosynthetic intermediates:
P-3
P-3A
P-3K, P-4
P-5, P-5m
P-6m, P-6mo

Fig. 1B

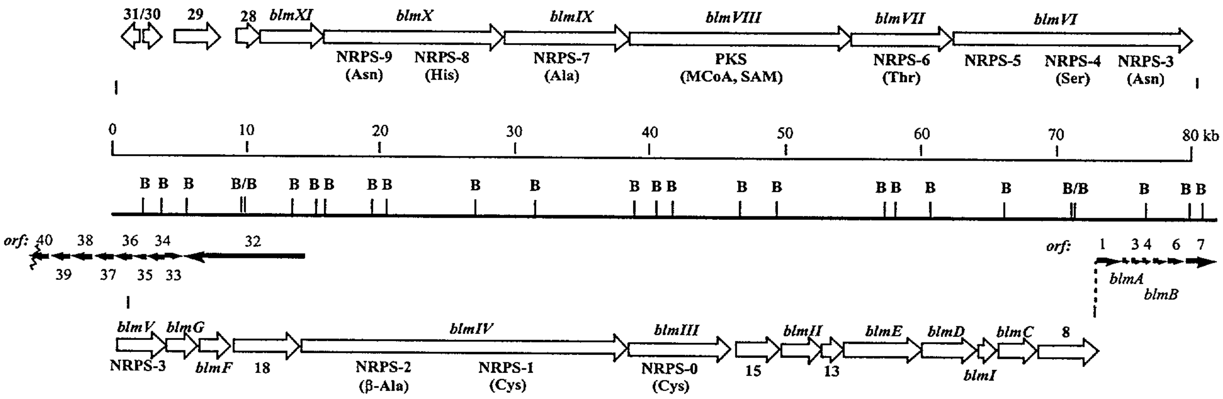

31/30
29
28
blmXI
blmX
blmIX
blmVIII
blmVII
blmVI
NRPS-9 (Asn)
NRPS-8 (His)
NRPS-7 (Ala)
PKS (MCoA, SAM)
NRPS-6 (Thr)
NRPS-5
NRPS-4 (Ser)
NRPS-3 (Asn)
0
10
20
30
40
50
60
70
80 kb
B B B B/B B B B B B B B B B B B B B B B B/B B B B
orf: 40 38 36 34 32
39 37 35 33
orf: 1 3 4 6 7
blmA
blmB
blmV
blmG
NRPS-3
blmF
18
blmIV
NRPS-2 (β-Ala)
NRPS-1 (Cys)
blmIII
NRPS-0 (Cys)
15
blmII
13
blmE
blmD
blmI
blmC
8

Fig. 2

Fig. 3A

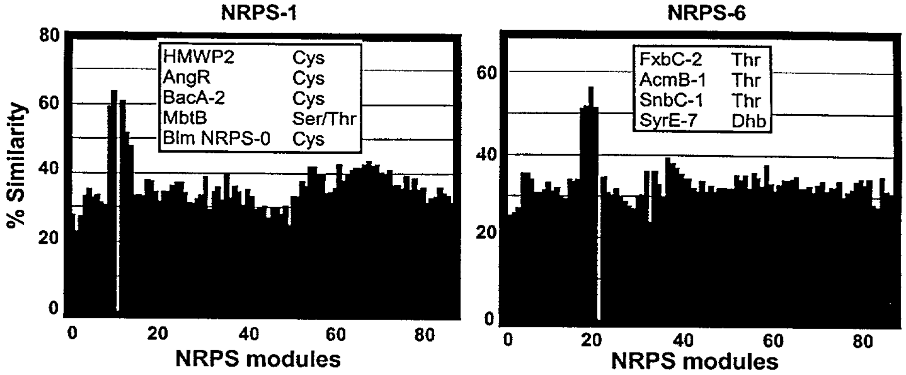
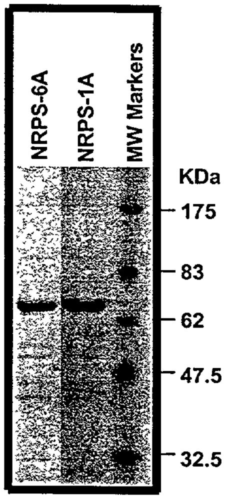


Fig. 3B

		Residues (PheA numbering) (16)							
NRPS module	Substrate	236	239	278	299	301	322	330	331
HMWP2	Cys	L	Y	N	M	S	M	I	W
AngR	Cys	L	Y	N	M	S	M	I	W
BacA-2	Cys	L	Y	N	L	S	L	I	W
MbtB	Ser/Thr	M	L	N	A	G	L	V	H
Blm NRPS-0	**Cys**	L	Y	H	L	G	L	P	W
Blm NRPS-1	**Cys**	L	Y	N	L	S	L	I	W
SyrE-7	Dhb	F	W	N	V	G	M	V	H
AcmB-1	Thr	F	W	N	V	G	M	V	H
SnbC-1	Thr	F	W	N	I	G	M	V	H
FxbC-2	Thr	F	W	N	V	G	M	V	H
Blm NRPS-6	**Thr**	F	W	S	V	G	M	I	H



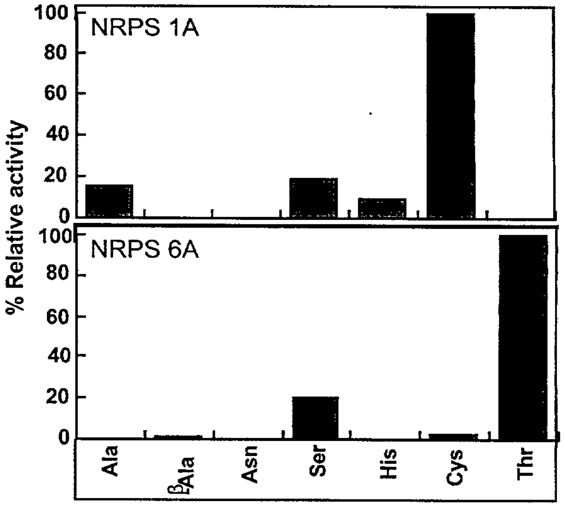


Fig. 3C

Fig. 3D

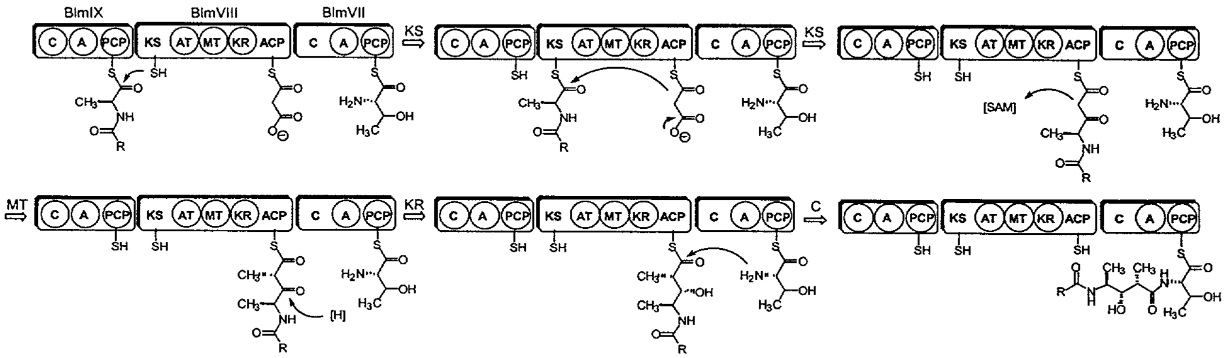
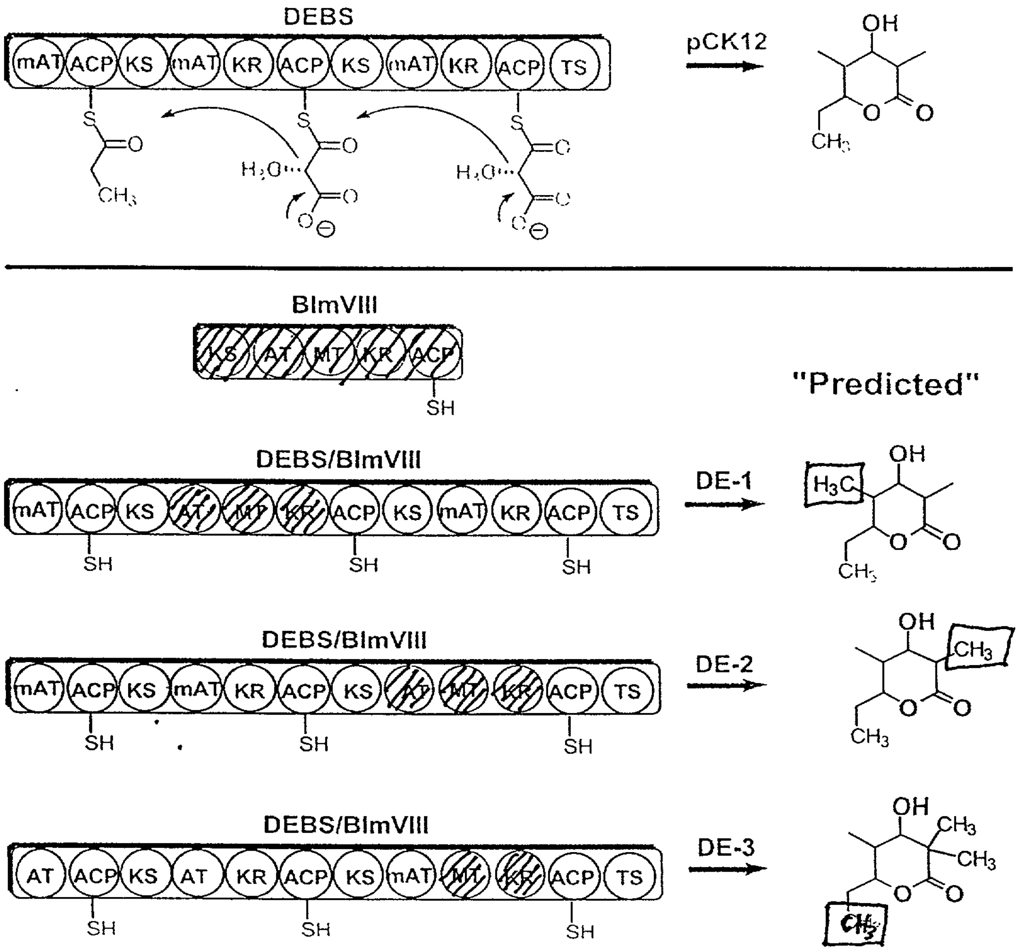

BlmIX
BlmVIII
BlmVII
C
A
PCP
KS
AT
MT
KR
ACP
KS
KS
[SAM]
MT
KR
C
[H]
SH
S
CH_3
NH
O
R
$O^{\ominus}$
H_2N
OH
H_3C
HO

Fig. 4


DEBS
mAT
ACP
KS
mAT
KR
ACP
KS
mAT
KR
ACP
TS
pCK12
OH
O
O
CH_3
S
O
CH_3
S
O
H_2O
O
O
S
O
H_2O
O
O
BlmVIII
KS
AT
MT
KR
ACP
SH
"Predicted"
DEBS/BlmVIII
mAT
ACP
KS
AT
MT
KR
ACP
KS
mAT
KR
ACP
TS
SH
SH
SH
DE-1
H_3C
OH
O
O
CH_3
DEBS/BlmVIII
mAT
ACP
KS
mAT
KR
ACP
KS
AT
MT
KR
ACP
TS
SH
SH
SH
DE-2
OH
CH_3
O
O
CH_3
DEBS/BlmVIII
AT
ACP
KS
AT
KR
ACP
KS
mAT
MT
KR
ACP
TS
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SH
SH
DE-3
OH
CH_3
CH_3
O
O
CH_3

Fig. 5

Fig. 6A

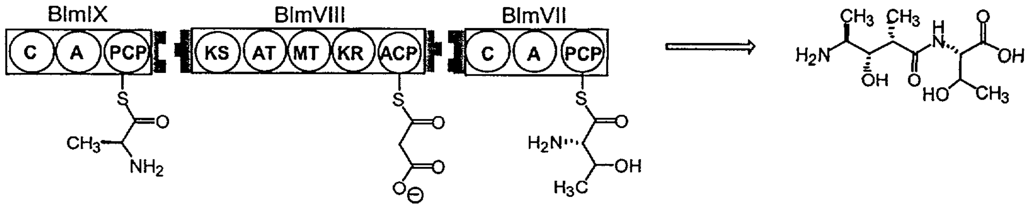


Fig. 6B

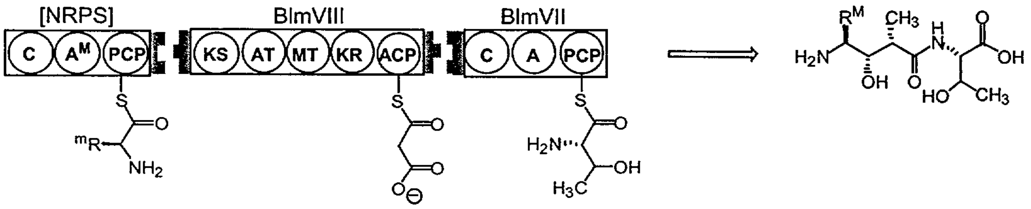


Fig. 6C

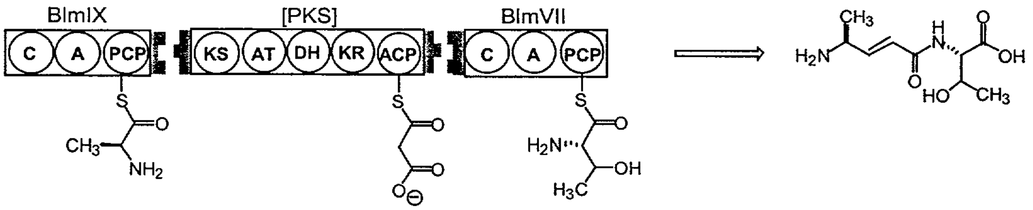


Fig. 6D

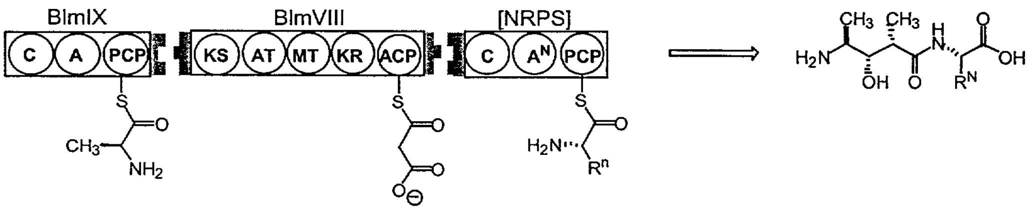


Fig. 6E

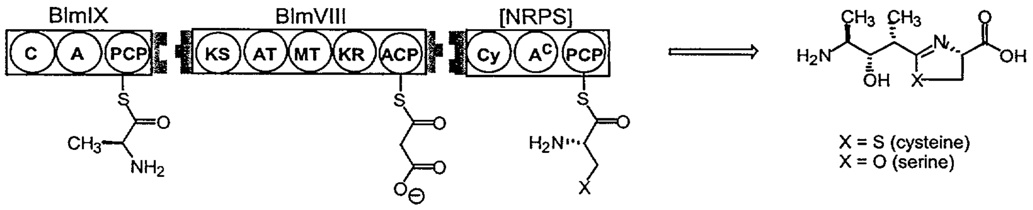
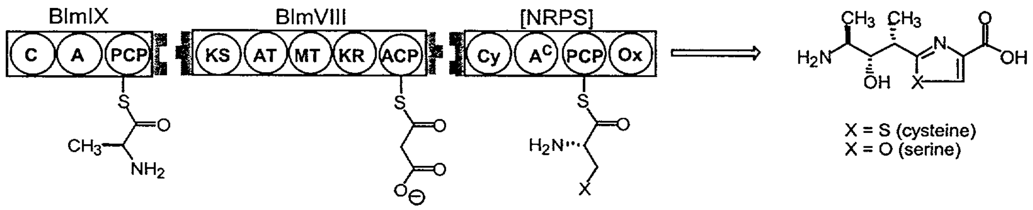


Fig. 6F



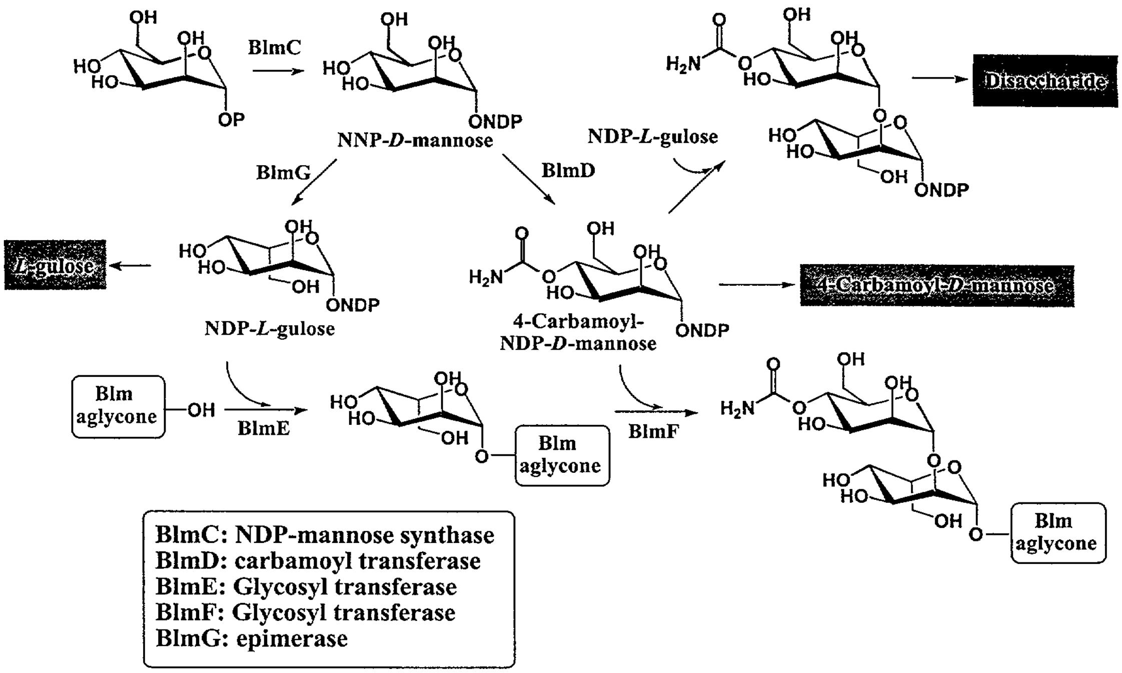
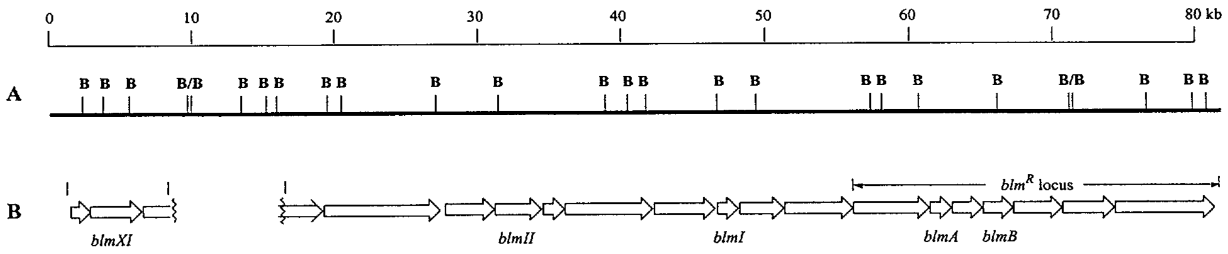

BlmC
NNP-D-mannose
ONDP
BlmG
BlmD
NDP-L-gulose
Disaccharide
L-gulose
NDP-L-gulose
4-Carbamoyl-
NDP-D-mannose
4-Carbamoyl-D-mannose
Blm aglycone
BlmE
BlmF
BlmC: NDP-mannose synthase
BlmD: carbamoyl transferase
BlmE: Glycosyl transferase
BlmF: Glycosyl transferase
BlmG: epimerase

Fig. 7



C

```
                                                                    -18    RBS
                                                                       CCGGACGGCGGCCCGCTC
ATGAGCGCCCCGCGGGGCGAGCGGACCCGGCGCCGCGCGCTCGAACGCGACATCGCCGCGATCTGGGCCGAGACCCTCGGCAGGGACAGCGTC  93
M  S  A  P  R  G  E  R  T  R  R  R  A  L  E  R  D  I  A  A  I  W  A  E  T  L  G  R  D  S  V
GGCCCGCACGAGGACTTCGCCGCGCTGGGCGGCAACTCCATCCACGCCATCAAGATCACCAACCGGGTGGAGGAACTCGTCGACGCCGAGCTG 186
G  P  H  E  D  F  A  A  L  G  G  N  S  I  H  A  I  K  I  T  N  R  V  E  E  L  V  D  A  E  L
TCCATCCGCGTCCTGCTCGAGACGCGCACCGTGGCCGGCATGACGGACCACGTCCACGCCACGCTCACGGGGGAGCGGGACCGGTGA      273
S  I  R  V  L  L  E  T  R  T  V  A  G  M  T  D  H  V  H  A  T  L  T  G  E  R  D  R  *
```

Fig. 8

```
Grs-2      3045-ISIGTEYVAPRTMLEGKLEEIWKDVLGLQRVGIHDDFFTIGGHSL-3089
Srfa-3      960-DQLAEEWIGPRNEMEETIAQIWSEVLGRKQIGIHDDFFALGGHSL-1004
Vir-S       557-GRSVEGRGVPRTPQQEILASLFAEVLGLSKVGIHEDFFDLGGHSL- 601
Saf-B      1661-LDPGQDYLAPRNELEARIAAIWEGLLRRERVGVHDSFFDLGGNSL-1705
BlmI          1-MSAPRGERTRRRALERDIAAIWAETLGRDSVGPHEDFAALGGNSI-  45
consensus     1-i  g eyvapR  le  ia iw evLgr rvGiHddFf lGGhSl-  45

Grs-2      3090-KAMAVISQVHKECQTEVPLRVLFETPTIQGLAKYIEETDTEQYMA-3134
Srfa-3     1005-KAMTAVPH.QQELGIDLPVKLLFEAPTIAGISAYLKNGGSDGLQD-1048
Vir-S       602-LATRLTSRIRTVLGAEIAVRDLFEAPTVEALAETLEEAREVRPAL- 646
Saf-B      1706-LATRLATRLAATLQVQAGVRTVFEHRTVAAQAAHFTQATKTHQAH-1750
BlmI         46-HAIKITNRVEELVDAELSIRVLLETRTVAGMTDHVHATLTGERDR-  90
consensus    46-kAmrv srv   l  ev vrvlfE pTvagla  i  g t     -  90
```

Fig. 9

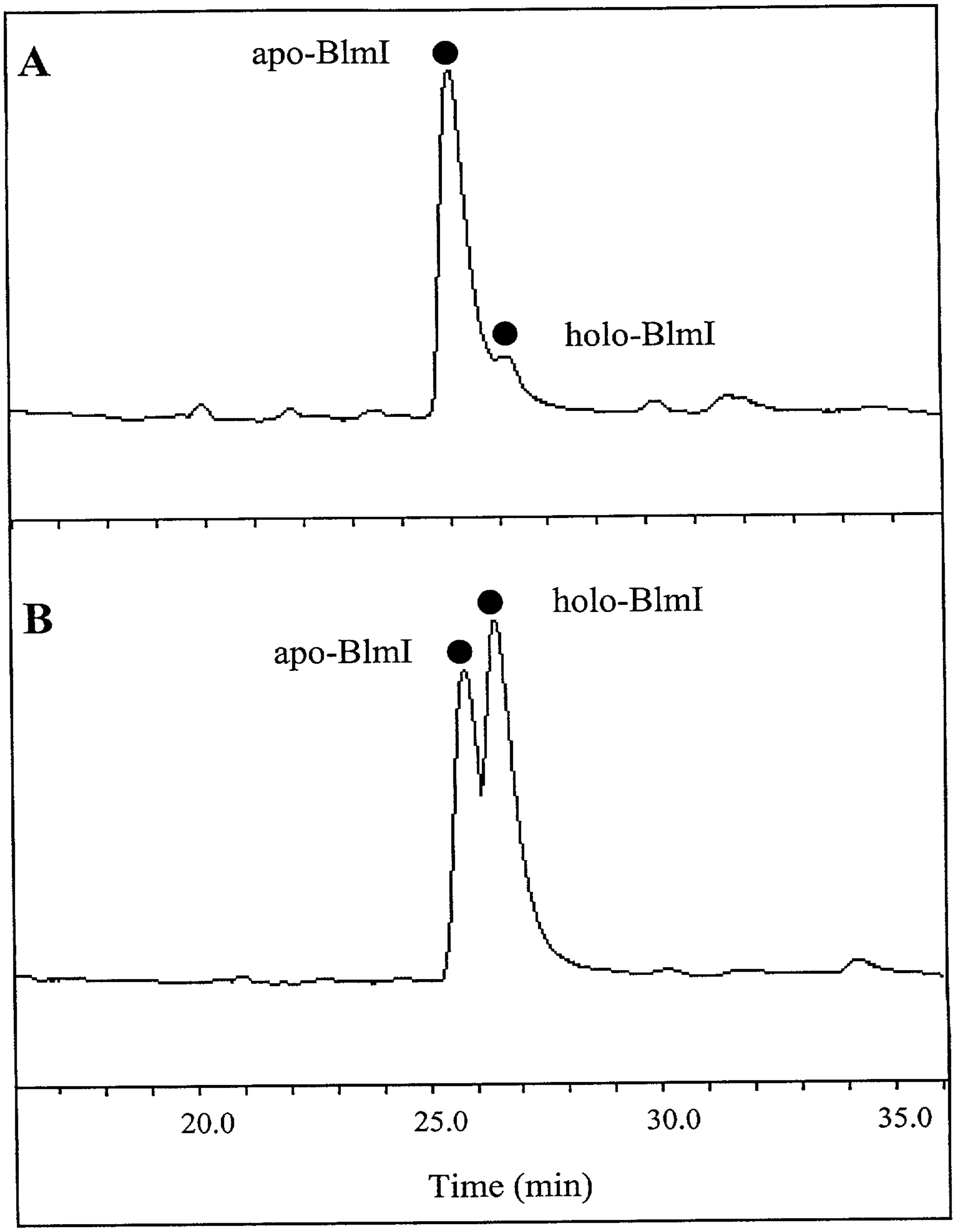

A
apo-BlmI
holo-BlmI
B
holo-BlmI
apo-BlmI
20.0
25.0
30.0
35.0
Time (min)

Fig. 10

Nonreiterative Type I Modular Protein Template

Fig. 11A

PKS

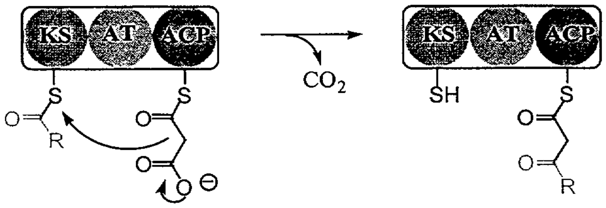
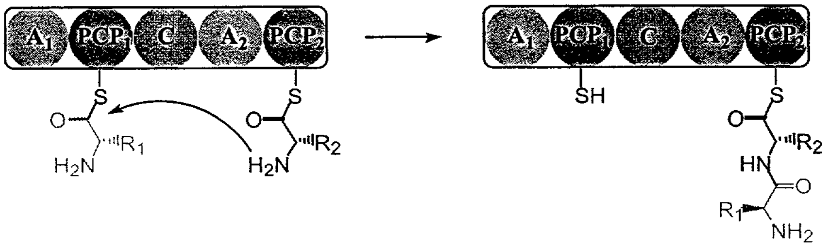


Fig. 11B

NRPS



Iterative Type II Protein Complex

Fig. 11C

PKS

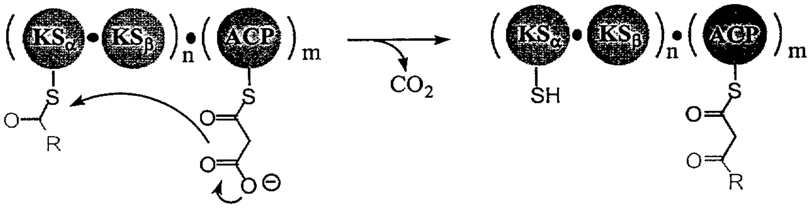
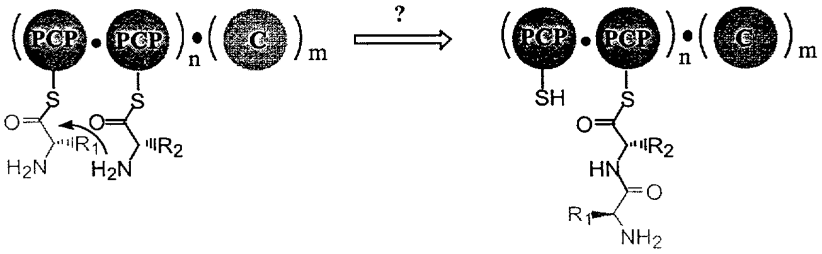


Fig. 11D

NRPS



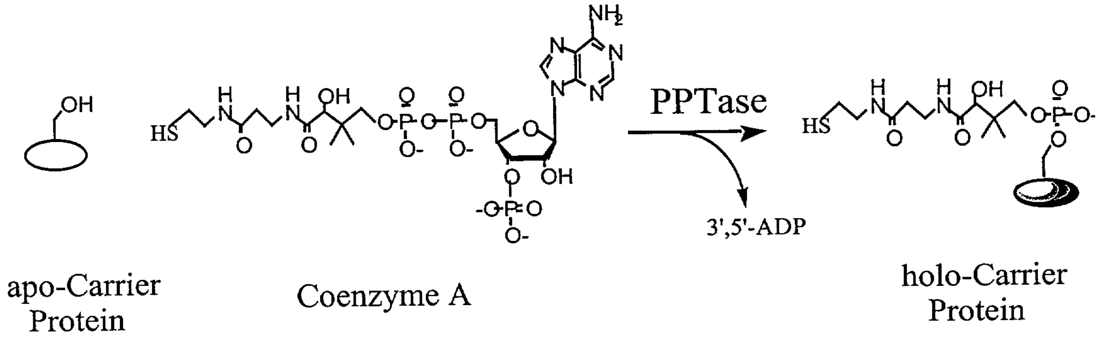

OH
HS
H N
O
H N
O
OH
O
O-P-O-P-O
O-
O-
NH2
N
N
N
N
O
OH
O
-O-P=O
O-
PPTase
3',5'-ADP
HS
H N
O
H N
O
OH
O
O-P-O-
O
apo-Carrier Protein
Coenzyme A
holo-Carrier Protein

Fig. 12

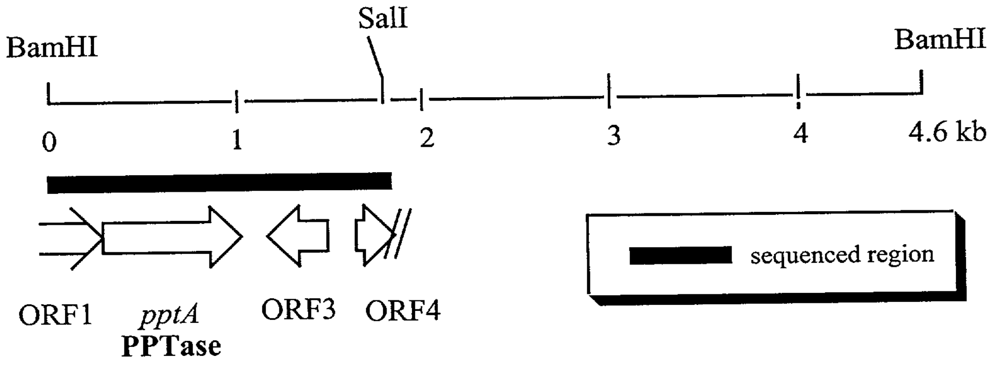

BamHI
SalI
BamHI
0
1
2
3
4
4.6 kb
sequenced region
ORF1
pptA
PPTase
ORF3
ORF4

Fig. 13

PATENT APPLICATION DECLARATION

(Attorney's Docket No.: 2500.125US2)

Each of the Applicants named below hereby declares as follows:

1. My residence, post office address and country of citizenship given below are true and correct.

2. I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought in the patent application entitled "BLEOMYCIN GENE CLUSTER COMPONENTS AND THEIR USES," Serial No. _______________, filed January 5, 2000, and I have reviewed and understand the contents of the specification, including its claims.

3. I acknowledge my duty to disclose to the Office all information known to me to be material to patentability of this application, in accordance with 37 C.F.R. Section 1.56, which is defined on the attached page.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: __________________ ____________________________________

Ben Shen
Residence and Post Office Address: 1842 Rushmore Lane
Davis, California 95616
(Citizenship: People's Republic of China)

Date: __________________ ____________________________________

Liangcheng Du
Residence and Post Office Address: 1301 Orchard Park Q-9
Davis, California 95616
(Citizenship: Peoples Republic of China)

Date: ____________________ ____
Cesar Sanchez

Residence and
Post Office Address:
(Citizenship: Spain)

Date: ____________________ ____
Mei Chen

Residence and
Post Office Address:
1301 Orchard Park Q-9
Davis, California 95616
(Citizenship: Peoples Republic of China)

Date: ____________________ ____
Daniel J. Edwards

Residence and
Post Office Address:
425 Russell Park, Apt. 4
Davis, California 95616
(Citizenship: United States)

Section 1.56 Duty to Disclose Information Material to Patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office, or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application;

(2) Each attorney or agent who prepares or prosecutes the application; and

(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

SEQUENCE LISTING

SEQ ID NO: 1 BLM gene cluster ORFS 30 through 8

(note orf 31-40 on sequence 1-18660 are translated on the reverse strand and on a separate file)

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18601   ACCCATCTCATAGGTGTACGCGCTGGAGCATTCGGGGCACGACGGAAGGTTCTCGGTCAC   18660
                 .         .         .         .         .         .
18661   GAGAGCACTGTAAGCCCGAACCCGCAAGGATGACGAATTGCAAAATTGTGCAAGTCGCTA   18720
                 .         .         .         .         .         .
18721   CATGATGGTCCGGCTGTGCCCGCAGGTAGCCGCGGGCACAGCACCAGACGCTGCCTCCGC   18780
                 .         .         .         .         .         .
18781   GCACCGCGCGGGAGGCCCGGTGAGGCGAGAGGCTGAGGTTCCGTGCCGGTTCCGCTGTAT   18840
                                                  M  P  V  P  L  Y     (orf30)
                 .         .         .         .         .         .
18841   CAGGCGAAGGCCGAGTTCTTCCGGATGCTGGGGCACCCGGTCCGCATCCGCGTACTGGAG   18900
        Q  A  K  A  E  F  F  R  M  L  G  H  P  V  R  I  R  V  L  E
                 .         .         .         .         .         .
18901   CTGCTGCAGGACGGGCCGATGCCGGTGCGTGATCTGCTGGCGGCGATCGAGATCGAGCCC   18960
        L  L  Q  D  G  P  M  P  V  R  D  L  L  A  A  I  E  I  E  P
                 .         .         .         .         .         .
18961   TCGGCGCTGTCCCAGCAGCTGGCGGTGTTGCGCCGCTCGGGCATCGTGACCTCCACCCGC   19020
        S  A  L  S  Q  Q  L  A  V  L  R  R  S  G  I  V  T  S  T  R
                 .         .         .         .         .         .
19021   ACGGGTTCCACGGTCGTCTACGAGCTGGCCGGTGGCGACGTGGCGGAGCTGATGTCCGCC   19080
        T  G  S  T  V  V  Y  E  L  A  G  G  D  V  A  E  L  M  S  A
                 .         .         .         .         .         .
19081   GCGCGCCGCATCCTGACCGAGATGCTCAATGGGCAGCACGAGCTGCTGGAGGAGCTGAGG   19140
        A  R  R  I  L  T  E  M  L  N  G  Q  H  E  L  L  E  E  L  R
                 .         .         .         .         .         .
19141   GAAGCCGAGGTCAGTGCCCGGTGAGCTCCCTCGCCGTCCGGGTGGGAGCCCGGGTGCGTT   19200
        E  A  E  V  S  A  R  *
                            M  S  S  L  A  V  R  V  G  A  R  V  R  S   (orf29)
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19201   CCGTGCTGCCCACCCGCGCCGACCTCGCGGGCATGGGCCGCAGCCCGCGACGTGATCTAC   19260
          V  L  P  T  R  A  D  L  A  G  M  G  R  S  P  R  R  D  L  L
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19261   TGGCCGGTCTGACCGTGGCGATCGTGGCCCTGCCGCTCGCCCTCGGATTCGGCGTCTCCT   19320
          A  G  L  T  V  A  I  V  A  L  P  L  A  L  G  F  G  V  S  S
                 .         .         .         .         .         .
19321   CCGGTCTCGGCGCGGAGGCAGGGCTGGCCACCGCGGTGGTGGCGGGCGCGCTGGCCGCGG   19380
          G  L  G  A  E  A  G  L  A  T  A  V  V  A  G  A  L  A  A  V
                 .         .         .         .         .         .
19381   TATTCGGTGGGTCGAATCTCCAGGTGTCCGGGCCCACGGGCGCCATGACCGTGGTCCTGG   19440
          F  G  G  S  N  L  Q  V  S  G  P  T  G  A  M  T  V  V  L  V
                 .         .         .         .         .         .
19441   TGCCCATCGTCGCCCGGTACGGCCCCGGCGGTGTCCTCACGGTCGGCCTGCTCGCCGGAC   19500
          P  I  V  A  R  Y  G  P  G  G  V  L  T  V  G  L  L  A  G  L
                 .         .         .         .         .         .
19501   TGATGCTGATCGCGCTCGCCCTCGCCCGCGCCGGCCGCTACATGCAGTACGTGCCGGCCC   19560
          M  L  I  A  L  A  L  A  R  A  G  R  Y  M  Q  Y  V  P  A  P
                 .         .         .         .         .         .
19561   CGGTGGTGGAGGGCTTCACCCTCGGCATCGCCTGCGTGATCGGCTTGCAGCAGGTGCCGA   19620
          V  V  E  G  F  T  L  G  I  A  C  V  I  G  L  Q  Q  V  P  N
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19621   ACGCCCTGGGAGTCGCCAAGCCGGAGGGCGACAAGGTCCTCGTCGTGACCTGGCGCGCGG   19680
          A  L  G  V  A  K  P  E  G  D  K  V  L  V  V  T  W  R  A  V
                 .         .         .         .         .         .
19681   TCGAGACCTTCGCCGGGGCGCCCAACTGGACCGCTGCCGGACTGGCGGCAGCGGTCGCCG   19740
          E  T  F  A  G  A  P  N  W  T  A  A  G  L  A  A  A  V  A  A
                 .         .         .         .         .         .
19741   CGGTCATGCTGACCGGCGCGCGGTGGCGGCCGGTCGTTCCCTTCTCCCTCCTCGCGGTGA   19800
          V  M  L  T  G  A  R  W  R  P  V  V  P  F  S  L  L  A  V  T
                 .         .         .         .         .         .
19801   CCGGTGCCACCGTCGTGGCCCAGCTGTGCCACCTGGACGCGGCCCGCCCGATCGGGGACC   19860
          G  A  T  V  V  A  Q  L  C  H  L  D  A  A  R  P  I  G  D  L
                 .         .         .         .         .         .
19861   TGCCCGCGGGGCTGCCCGCCCCGTCGCTGGCCTTCCTGGACCTCGGAGCACTGGGCTCGC   19920
          P  A  G  L  P  A  P  S  L  A  F  L  D  L  G  A  L  G  S  L
                 .         .         .         .         .         .
19921   TGCTGGCGCCTGCCGTGGCCGTGGCGGCCCTTGCCGCGTTGGAATCGCTGCTGTCGGCGT   19980
          L  A  P  A  V  A  V  A  A  L  A  A  L  E  S  L  L  S  A  S
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                .         .         .         .         .         .
19981  CCGTCGCGGACGGCATGACGGTCGGGCAGAAGCACGACCCGGACAAGGAGCTGTTCGGGC   20040
        V  A  D  G  M  T  V  G  Q  K  H  D  P  D  K  E  L  F  G  Q
                .         .         .         .         .         .
20041  AGGGTCTCGCCAACCTGGCCGCCCCGCTGTTCGGCGGCGTCCCGGCCACCGGCGCGATAG   20100
        G  L  A  N  L  A  A  P  L  F  G  G  V  P  A  T  G  A  I  A
                .         .         .         .         .         .
20101  CCCGCACCGCCGTCAACGTCCGTACCGGTGCGAGCTCGCGACTGGCGGCCCTCACGCACG   20160
        R  T  A  V  N  V  R  T  G  A  S  S  R  L  A  A  L  T  H  A
                .         .         .         .         .         .
20161  CCGCGATCCTCGCCGTCATCGTCTTCGCCGCCGCCCCACTGGTCTCCCGCATCCCCCTGG   20220
        A  I  L  A  V  I  V  F  A  A  A  P  L  V  S  R  I  P  L  A
                .         .         .         .         .         .
20221  CCGCGCTCGCCGGCGTGCTGATCGCGACCGCGATCCGCATGGTCGAAGTGGGCAGCCTGC   20280
        A  L  A  G  V  L  I  A  T  A  I  R  M  V  E  V  G  S  L  R
                .         .         .         .         .         .
20281  GGGCGATGGCCCGCGCCACGCGCTCCGACGGCCTGGTACTGATCCTCACGGCGGTCGCCA   20340
        A  M  A  R  A  T  R  S  D  G  L  V  L  I  L  T  A  V  A  T
                .         .         .         .         .         .
20341  CCGTGGCCCTGGACCTCGTCTACGCCGTCATCATCGGCCTGCTGGTCGCCGGCGCACTCG   20400
        V  A  L  D  L  V  Y  A  V  I  I  G  L  L  V  A  G  A  L  A
                .         .         .         .         .         .
20401  CCCTGCGGGCCGTGGCCAAGCAGGTCCGCCTGGACCAGGTCTCCTTGAAGGAGGACCTGA   20460
        L  R  A  V  A  K  Q  V  R  L  D  Q  V  S  L  K  E  D  L  T
                .         .         .         .         .         .
20461  CCGGCGACCACAGCGCCGAGGAACACGCGCTGCTCGCCGAGCACATCGTGGCGTACCGCA   20520
        G  D  H  S  A  E  E  H  A  L  L  A  E  H  I  V  A  Y  R  I
                .         .         .         .         .         .
20521  TCGACGGTCCGCTGTTCTTCGCCGCGGCCCACCGCTTCCTGCTGGAACTCTCGGACGTCG   20580
        D  G  P  L  F  F  A  A  A  H  R  F  L  L  E  L  S  D  V  A
                .         .         .         .         .         .
20581  CGGACGTGCGCGTGGTGATCCTGCGCATGTCCCGCGTGACCACCATGGACGCCACCGGCG   20640
        D  V  R  V  V  I  L  R  M  S  R  V  T  T  M  D  A  T  G  A
                .         .         .         .         .         .
20641  CCCTCGTCCTGAAGGACGCGGTCACCAAGCTGAACCGGCGCGGCATCACCGTCCTGGCCT   20700
        L  V  L  K  D  A  V  T  K  L  N  R  R  G  I  T  V  L  A  S
                .         .         .         .         .         .
20701  CCGGGGTACGCCCCGGCCAGCGCCGGGTCCTCGACTCCGTCGGCGCCCTCGGTCTGCTCC   20760
        G  V  R  P  G  Q  R  R  V  L  D  S  V  G  A  L  G  L  L  R
                .         .         .         .         .         .
20761  GGGCCGCCACCGGCGACGACTACACCGGCACTCCCGAAGCCATCGCCGCCGCCCGAAGCC   20820
        A  A  T  G  D  D  Y  T  G  T  P  E  A  I  A  A  A  R  S  H
                .         .         .         .         .         .
20821  ACCTGCACGGCGCCGGTGTCCTGGCCCCCGCCTGCCCGGGCCCGCCTCCTCCGGTACCCC   20880
        L  H  G  A  G  V  L  A  P  A  C  P  G  P  P  P  P  V  P  P
                .         .         .         .         .         .
20881  CACCGTGCGCTCCGAGTGCCCGACGATGAGGAGCCGACCGAGGTCCTCCTCCGTCACCCG   20940
        P  C  A  P  S  A  R  R  *
                .         .         .         .         .         .
20941  GACACCCACGGTTGCGCCGCCCCATGCCGGCGGTCCCTCCTGACGGCCCGTCCGCGGCTT   21000
                .         .         .         .         .         .
21001  GAGGCGGCGGTGGACGGCCTGCCGCCGCCGGCCTCGGGCTGATCGGCGTGATCACCGCCC   21060
                .         .         .         .         .         .
21061  ATGCGCGGGTGGGCGCCCGCGGCATCGTGGGCGGACCGTGTTCCCGGCCACCGCGGCGG   21120
                .         .         .         .         .         .
21121  CCGGCCTCGCGCTGGGCGTGGCCTGCCGCGGTGCCTGGTAGCGGCGGGGTCCGGCGGCCG   21180
                .         .         .         .         .         .
21181  GGCCTGTGCTTCTTCCCGCCCGTCCGGCGGGTGGCGCCGCGCCGGCGGTGACAGGGAAAT   21240
                .         .         .         .         .         .
21241  ATGACCGGAACTGGGATGCTCGCGTCCACTCGGGTGTGTTTAAGTGCCACGGGGGCTTCC   21300
                .         .         .         .         .         .
21301  GACGGCGCGTCGCGCGCCGGCGGTTCGCCCGATGATGGTCGTGCGGCGCTGTGAGCCGGG   21360
                .         .         .         .         .         .
21361  GAGCCTATGGCACAGGACCTGAACGACTGGATCGAGGACGAGGTCGTCCCTTACGAGGAG   21420
              M  A  Q  D  L  N  D  W  I  E  D  E  V  V  P  Y  E  E    (orf28)
                .         .         .         .         .         .
21421  AAGCCTCTCGAATGGATCTCCCAGTACCACTTCTTCCGCGACCCGGCGCGAGCCGCCTAT   21480
        K  P  L  E  W  I  S  Q  Y  H  F  F  R  D  P  A  R  A  A  Y
                .         .         .         .         .         .
21481  GTCGATCACACCTACTTCTTCTCACCGGCCGATGGCGCGATCGTCTACCAGAAAGTAGTG   21540
        V  D  H  T  Y  F  F  S  P  A  D  G  A  I  V  Y  Q  K  V  V
                .         .         .         .         .         .
21541  GATCCCCAGGAGTCGATCATCGACATCAAGGGGAAGCCGTACTCGCTGGCCGCCGCGCTC   21600
        D  P  Q  E  S  I  I  D  I  K  G  K  P  Y  S  L  A  A  A  L
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                .         .         .         .         .         .
21601  CGTGACGAATCGTTCGGTCACCGGTGCCTGGTGATCGGCATCTTCATGACCTTCTTCGAC   21660
       R  D  E  S  F  G  H  R  C  L  V  I  G  I  F  M  T  F  F  D
                .         .         .         .         .         .
21661  GTGCACATCAACCGGATGCCTTACGGCGGCCGTCTCTCCTTCGCGCTCAAGGAGCCCATC   21720
       V  H  I  N  R  M  P  Y  G  G  R  L  S  F  A  L  K  E  P  I
                .         .         .         .         .         .
21721  GGGACGTTCAACCTCCCCATGCTGGCCATGGAGCAGGACCTGCTCGAACGGCTCCGGGTC   21780
       G  T  F  N  L  P  M  L  A  M  E  Q  D  L  L  E  R  L  R  V
                .         .         .         .         .         .
21781  AATCCGGCTCACGCGAGGTATCTGCACCTGAACGAGCGGATGGTCAACCGGGTCGACGCG   21840
       N  P  A  H  A  R  Y  L  H  L  N  E  R  M  V  N  R  V  D  A
                .         .         .         .         .         .
21841  CCGCGGCTCCGGGGCCCGTACTGGATGCTCCAGATCGCCGACTACGACGTCGACTCCATC   21900
       P  R  L  R  G  P  Y  W  M  L  Q  I  A  D  Y  D  V  D  S  I
                .         .         .         .         .         .
21901  ACCCCGTTCTGCAGACGGCAGGGAATGTTCCGCTCCCAGGGGCGCCGCTTCTCCCAGATC   21960
       T  P  F  C  R  R  Q  G  M  F  R  S  Q  G  R  R  F  S  Q  I
                .         .         .         .         .         .
21961  CGCTACGGATCGCAGGTCGACCTGGTGATCCCGATGGCGGCCGACCGCGAGTACGTCCCC   22020
       R  Y  G  S  Q  V  D  L  V  I  P  M  A  A  D  R  E  Y  V  P
                .         .         .         .         .         .
22021  GTGGAGGCCGTCGGCCGGCACGTGAAGGCGGGGCTCGACCCGCTCGTCAAGATCCGGTGG   22080
       V  E  A  V  G  R  H  V  K  A  G  L  D  P  L  V  K  I  R  W
                .         .         .         .         .         .
22081  CGTTGAAGAGCGCGTACGAAGCGATGGCGAACTGGAGGGACACAGCGTGGGTTTCCGTCG   22140
       R  *                                          M  G  F  R  R   (orf27)
                .         .         .         .         .         .
22141  AGCGCAGAGGGCCGGTGGGCCGGGAGCGGGCCGGCGGGAGAGCGCCCGGTTCAGGCCGGA   22200
        A  Q  R  A  G  G  P  G  A  G  R  R  E  S  A  R  F  R  P  D
                .         .         .         .         .         .
22201  CGGGCCGTCGGCGCCGCGGGACCGTCCGTTACCCCTGTCCGCCGGGCAGTTGTTCGAGTG   22260
        G  P  S  A  P  R  D  R  P  L  P  L  S  A  G  Q  L  F  E  W
                .         .         .         .         .         .
22261  GGTGTTTGACAAGCTCGTCGACGGAGATCTGAGCCACCAGCCGACGATTGTGCGGCTCCG   22320
        V  F  D  K  L  V  D  G  D  L  S  H  Q  P  T  I  V  R  L  R
                .         .         .         .         .         .
22321  CGGCCCGCTGAACACCGCCGCCCTGCGGATGGCCTACGCCCGGCTGGTGCGGCGCCACGA   22380
        G  P  L  N  T  A  A  L  R  M  A  Y  A  R  L  V  R  R  H  E
                .         .         .         .         .         .
22381  GTGCCTGCGCACCCGCTTCCCCGTGATCGACGGGGAGCCCGTGCAGGTGATCGAGGGCAT   22440
        C  L  R  T  R  F  P  V  I  D  G  E  P  V  Q  V  I  E  G  I
                .         .         .         .         .         .
22441  CGGGAAAGCAGCGGGGGGCCCGCTGCCGCTCATCGATCTGCGCCACCTCCCGGAGGCGCT   22500
        G  K  A  A  G  G  P  L  P  L  I  D  L  R  H  L  P  E  A  L
                .         .         .         .         .         .
22501  TCGCGCGCGCGAGATCGCGAGGATCCGCGAGGAGACGCTGTCCACGCCGGTCCCCTTCGA   22560
        R  A  R  E  I  A  R  I  R  E  E  T  L  S  T  P  V  P  F  D
                .         .         .         .         .         .
22561  CAAGCGGCCGCCCGTCCGCGTGGCGCTGATCCGGGCGGCGCCCGAGGAGCACCTCTTCCT   22620
        K  R  P  P  V  R  V  A  L  I  R  A  A  P  E  E  H  L  F  L
                .         .         .         .         .         .
22621  CGTCGGCATCCCGCACATCACCGCGGACCTGTGGTCCGCGACCCTGCTCAACGACGAGCT   22680
        V  G  I  P  H  I  T  A  D  L  W  S  A  T  L  L  N  D  E  L
                .         .         .         .         .         .
22681  CATGGCGCACTACAGGGCGGGGGCCGAGGGGACTCCCTCCCGGGCCCCCACCCCCGTCGC   22740
        M  A  H  Y  R  A  G  A  E  G  T  P  S  R  A  P  T  P  V  A
                .         .         .         .         .         .
22741  GCAGTACGCCGACTTCGCGCAGTGGCAGCGCGCGTGGTGGAACCGGGACCGCACCGAGCG   22800
        Q  Y  A  D  F  A  Q  W  Q  R  A  W  W  N  R  D  R  T  E  R
                .         .         .         .         .         .
22801  GGAGGCCGGACGGTGGCGGGCGCGGCTGGACGGGCTGTCCGCCGTGGAACTGCCCCTGGA   22860
        E  A  G  R  W  R  A  R  L  D  G  L  S  A  V  E  L  P  L  D
                .         .         .         .         .         .
22861  CCGGCCCCGCCCCGCGGGCCGCCGGCGGGACTGCTTCCTGATCGGGGACACCTTCGACGC   22920
        R  P  R  P  A  G  R  R  R  D  C  F  L  I  G  D  T  F  D  A
                .         .         .         .         .         .
22921  CGAACTGAGCGACCGGCTGCGCGCCTTGGCACGCACCGCCGACGTCACGCTGTACGTGGT   22980
        E  L  S  D  R  L  R  A  L  A  R  T  A  D  V  T  L  Y  V  V
                .         .         .         .         .         .
22981  GCTGCTGGCGGCGTTCCACTGGCTGGTGGGGCGGATGTCGGGCGCCGGCCGGCTGGTGAC   23040
        L  L  A  A  F  H  W  L  V  G  R  M  S  G  A  G  R  L  V  T
                .         .         .         .         .         .
23041  CACCTCGCTCGTGGCCGCCCGGCACGGCAGCGCGGTACAGGGGATGACCGGCCCGTTCTC   23100
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         T  S  L  V  A  A  R  H  G  S  A  V  Q  G  M  T  G  P  F  S
                 .         .         .         .         .         .
23101   GGACTACCTGGCCCTGGTCGGGGACCTGTCGGGCGATCCGGACTTCCTGGAGTCCCTGCG   23160
         D  Y  L  A  L  V  G  D  L  S  G  D  P  D  F  L  E  S  L  R
                 .         .         .         .         .         .
23161   CCGCGTACGCGACGAGTGCCTGACCGCCCACGACCACCAGCGGCTTCCGTTCTCACAGGT   23220
         R  V  R  D  E  C  L  T  A  H  D  H  Q  R  L  P  F  S  Q  V
                 .         .         .         .         .         .
23221   CCTCGAAGTCATGGACCCCGGACGCGAGTTGCACCCCCATCCGCTGGAGCAGCTCGGGTT   23280
         L  E  V  M  D  P  G  R  E  L  H  P  H  P  L  E  Q  L  G  F
                 .         .         .         .         .         .
23281   CAACCTCCACAACATCCCTCCCGCGGTCATGGACTTCTCCGGCGACGTCGTCGTCTCGGC   23340
         N  L  H  N  I  P  P  A  V  M  D  F  S  G  D  V  V  V  S  A
                 .         .         .         .         .         .
23341   GGTGAACCCGGAGGGGGACGACGGGGAGAGCGGCGACGGGGAGTACGTGCCCTGGACCGC   23400
         V  N  P  E  G  D  D  G  E  S  G  D  G  E  Y  V  P  W  T  A
                 .         .         .         .         .         .
23401   CGACCTGACCTTCGACGTCTACGACTACGGCACCGGCCATATGCCGTTCGACGTGATACT   23460
         D  L  T  F  D  V  Y  D  Y  G  T  G  H  M  P  F  D  V  I  L
                 .         .         .         .         .         .
23461   CGACCGGCGGCTGGCCGATCCGGCGACGGCCCGGGAGTGGGCCGGGCACTACCGGTCGGT   23520
         D  R  R  L  A  D  P  A  T  A  R  E  W  A  G  H  Y  R  S  V
                 .         .         .         .         .         .
23521   GCTCCGTGCGGTCGTCGCCGACCCCGGCGTGCGCCTGTCCGCCCTCGGCACCCTGCTGTC   23580
         L  R  A  V  V  A  D  P  G  V  R  L  S  A  L  G  T  L  L  S
                 .         .         .         .         .         .
23581   CCTGCCGCGACCGCCGTCCGCCACGTCCTTCGGCGGCCGGGAGATCGACGTCCGGCGCGT   23640
         L  P  R  P  P  S  A  T  S  F  G  G  R  E  I  D  V  R  R  V
                 .         .         .         .         .         .
23641   CGAACGCGAGTTGGCGGGGCGCGACGGGATCACCGCCGCCCTGGTCGCGGTGGCGCCCCG   23700
         E  R  E  L  A  G  R  D  G  I  T  A  A  L  V  A  V  A  P  R
                 .         .         .         .         .         .
23701   GCGCCTGGCCACCGGGCTGCGCGTACGGGAACTGGTCGCCTACTGCGCCGTCGAGGGCAC   23760
         R  L  A  T  G  L  R  V  R  E  L  V  A  Y  C  A  V  E  G  T
                 .         .         .         .         .         .
23761   GCCGCGTCCGAACGCGGCCCACGACATCCGCGGCCGCCTGCGGGAGCGCCTGCCCGACGG   23820
         P  R  P  N  A  A  H  D  I  R  G  R  L  R  E  R  L  P  D  G
                 .         .         .         .         .         .
23821   CTGGGTGCCGACCGTGTTCGTCGAGCGCCCGCCGGAGGAGATCCGGAAGGCCCTGGCCGC   23880
         W  V  P  T  V  F  V  E  R  P  P  E  E  I  R  K  A  L  A  A
                 .         .         .         .         .         .
23881   CCGGGCGGCGGGCGGCGAACGGGCGGAGCCGCTGCCGCCGCCCGAGGACTGCGTCCCGCT   23940
         R  A  A  G  G  E  R  A  E  P  L  P  P  P  E  D  C  V  P  L
                 .         .         .         .         .         .
23941   TCCCGAGGAGGGCCGGCCCCCCTCGGACCCGTCCGAGCGGCGGCTGGCCGCGCTCTGGGC   24000
         P  E  E  G  R  P  P  S  D  P  S  E  R  R  L  A  A  L  W  A
                 .         .         .         .         .         .
24001   CGAGATCCTGGGCGCCCCGCCGAAGAGCGTGACCGAGCCCTTCTTCCGCGTCGGCGTCAC   24060
         E  I  L  G  A  P  P  K  S  V  T  E  P  F  F  R  V  G  V  T
                 .         .         .         .         .         .
24061   CGATAAGGACGCCCTCCGCTTCCTGGCCCGCGTGGCGGAGGACTTCGGCGTCACCGTGCC   24120
         D  K  D  A  L  R  F  L  A  R  V  A  E  D  F  G  V  T  V  P
                 .         .         .         .         .         .
24121   CTTCGCCGACTTCCTCAGCGCTCCCAACCTGCGTATGGTGAAGGACAATTTGGCTGAGAA   24180
         F  A  D  F  L  S  A  P  N  L  R  M  V  K  D  N  L  A  E  K
                 .         .         .         .         .         .
24181   ACGGAGGGTGTAACGCGCAATGAGTGAGTGGTAGGGTCGGAATCGAACCGCACTGATCGG   24240
         R  R  V  *
                 .         .         .         .         .         .
24241   CAATCTTTTCGGTCAGCTGTTCCGGATATTCCGGGGCGCGTCGGCGCTCCCTCGACCAAG   24300
                 .         .         .         .         .         .
24301   GGCGTACGCGGATAAGCGTGCGCCGCCCCACGGCTGCGTCTCGACGCCTTCATCGGCGCG   24360
                 .         .         .         .         .         .
24361   TCGGACACTTCGCGGTGCCAGTCGGCACGCTCAGAGATCAGTGGAATGCCTCGGTGTGCC   24420
                                                     M  P  R  C  A      (orf26)
                 .         .         .         .         .         .
24421   CGAGGTGCGCTCAGTACTGCTGTCCACACAACGCGCCAAGGGAGTTGGAACGTGATGGAG   24480
        R  G  A  L  S  T  A  V  H  T  T  R  Q  G  S  W  N  V  M  E
                 .         .         .         .         .         .
24481   ACGGCGAATTCCGGCTATCGGGTCTCACCTCAGCAGCGGCATTTATGGGCCATGCTGACC   24540
        T  A  N  S  G  Y  R  V  S  P  Q  Q  R  H  L  W  A  M  L  T
                 .         .         .         .         .         .
24541   CGCGGGCGGGACGGCGGGCGACGTGCGTTCACCCAGTCCGCCGTGGTGGTCGACCGTTCC   24600
        R  G  R  D  G  G  R  R  A  F  T  Q  S  A  V  V  V  D  R  S
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                .         .         .         .         .         .
24601  CTGGACGCCGCACGTCTGCGCGCCGCGCTGGCCTCCGTGGTGGCCGCCCACGAGCCGCTG  24660
       L  D  A  A  R  L  R  A  A  L  A  S  V  V  A  A  H  E  P  L
                .         .         .         .         .         .
24661  CGGACGACCTTCACCGGTCTCGCGGACGGACCGCGCCGGTCCAGGTCGTCCATGACCCG  24720
       R  T  T  F  T  G  L  A  G  R  T  A  P  V  Q  V  V  H  D  P
                .         .         .         .         .         .
24721  GACGAGCAGCCGCTGTCCGTCGTCGACCTGCCGCCCTCGTGCGCCGACGGCTCGGGCCCG  24780
       D  E  Q  P  L  S  V  V  D  L  P  P  S  C  A  D  G  S  G  P
                .         .         .         .         .         .
24781  GAACTGGACGAGCTCCGGCTCCGCGAACGCGCCGCCCTCGACCCGCGCGGCGGGCCCGTC  24840
       E  L  D  E  L  R  L  R  E  R  A  A  L  D  P  R  G  G  P  V
                .         .         .         .         .         .
24841  TTCCGGGCCGCCCTGGCGCGGGCCGGCGAGGACCGGGCGGTGCTGGTGCTCACCGCGCAC  24900
       F  R  A  A  L  A  R  A  G  E  D  R  A  V  L  V  L  T  A  H
                .         .         .         .         .         .
24901  GCCCTGGTCGCGGACCGGCTCTCCCTCCGGCTGCTGGCCGGGCAGATCCTCGCGGCGTAC  24960
       A  L  V  A  D  R  L  S  L  R  L  L  A  G  Q  I  L  A  A  Y
                .         .         .         .         .         .
24961  AGCGGGGAGACCGTGTCCCCCGATGGCCCGCCGCCCTTGCAGTACGCCGACTTCGCCGCC  25020
       S  G  E  T  V  S  P  D  G  P  P  P  L  Q  Y  A  D  F  A  A
                .         .         .         .         .         .
25021  TGGCACCACGACCTGCTCACCGCCGAGGACGCCGCCCCCGACCGCGCGCACTGGGCCGCC  25080
       W  H  H  D  L  L  T  A  E  D  A  A  P  D  R  A  H  W  A  A
                .         .         .         .         .         .
25081  CACACCGCCACCGCCGGCACCGGGCCGCTCCCCGGCGTCGTACGGCCCGGCGCCGCCCCG  25140
       H  T  A  T  A  G  T  G  P  L  P  G  V  V  R  P  G  A  A  P
                .         .         .         .         .         .
25141  GGTCCGTGGCGGGCGCGGGAGTGGGAACTGCCCGCCGAACTGGTGGCGGGGATCGACGGC  25200
       G  P  W  R  A  R  E  W  E  L  P  A  E  L  V  A  G  I  D  G
                .         .         .         .         .         .
25201  GTCGCCGGGAAGCTGTCCACCGATCCCGCCACCGTGCTGCACGCCGCCTTCCGTATCGCG  25260
       V  A  G  K  L  S  T  D  P  A  T  V  L  H  A  A  F  R  I  A
                .         .         .         .         .         .
25261  GTCTGGCGGCTCGCCGGCGAGCGGAACCTGCCCGTCGCCCTCACTCGTGACGGCCGTTCC  25320
       V  W  R  L  A  G  E  R  N  L  P  V  A  L  T  R  D  G  R  S
                .         .         .         .         .         .
25321  CACCCCGAACTCCGCACCGCGATCGGCGCCTTCGAGCGTGAGCTCCCGCTCGTCCACGAG  25380
       H  P  E  L  R  T  A  I  G  A  F  E  R  E  L  P  L  V  H  E
                .         .         .         .         .         .
25381  ATCCGTCACGAGACGGCGTTCGCGGAATACGCGCGCGCTCTGGACGCGCTCGTCGCCGAG  25440
       I  R  H  E  T  A  F  A  E  Y  A  R  A  L  D  A  L  V  A  E
                .         .         .         .         .         .
25441  GGCGAGGAACTCCTCGACCATTGCGACCCGGAACTGCTCGGCAGCCTCGACGGCACCGCG  25500
       G  E  E  L  L  D  H  C  D  P  E  L  L  G  S  L  D  G  T  A
                .         .         .         .         .         .
25501  GAAGGGCCCTGCTTCACCTTCACCCACCACCAGGCCGAAACACCGGTCCGGCGGGCCGGC  25560
       E  G  P  C  F  T  F  T  H  H  Q  A  E  T  P  V  R  R  A  G
                .         .         .         .         .         .
25561  ATCACCTTTACCACCGTCCATCAGGATTCGGGTACGCCGATTCCCGTCCGCCTGACCGCC  25620
       I  T  F  T  T  V  H  Q  D  S  G  T  P  I  P  V  R  L  T  A
                .         .         .         .         .         .
25621  CGACGCGACGGCGCCCGGCTGCGCATGGAACTGGGATACGACGAGGGCCGTATCGACGAG  25680
       R  R  D  G  A  R  L  R  M  E  L  G  Y  D  E  G  R  I  D  E
                .         .         .         .         .         .
25681  ACGTTTCCCGAGAACGCCGCCGCCTGCCTCACCCGCATTCTCGAAGGCGTCGTCTCCGCC  25740
       T  F  P  E  N  A  A  A  C  L  T  R  I  L  E  G  V  V  S  A
                .         .         .         .         .         .
25741  CCCGAGGGCCCGGTCGGCGACATCCGCATGCTGTCGGACGAGACCGCACGGCTGCTCCGG  25800
       P  E  G  P  V  G  D  I  R  M  L  S  D  E  T  A  R  L  L  R
                .         .         .         .         .         .
25801  GAAGCGGGGCTGGGCCCCCGCGTGGAACTTCCCGGCAAGGCGGTCCACGAACTCTTCGCC  25860
       E  A  G  L  G  P  R  V  E  L  P  G  K  A  V  H  E  L  F  A
                .         .         .         .         .         .
25861  GAGCAGGCCGCGCGCACCCCCGGGGCGGTCGCGGTCAGCGCGGGCGAGGACGCCCTCACG  25920
       E  Q  A  A  R  T  P  G  A  V  A  V  S  A  G  E  D  A  L  T
                .         .         .         .         .         .
25921  TACGCCGAACTCGACGAGCGGTCCAACCGCCTGGCACACCACCTGACCGGGCTCGGGGTG  25980
       Y  A  E  L  D  E  R  S  N  R  L  A  H  H  L  T  G  L  G  V
                .         .         .         .         .         .
25981  ACACCCGGCCGGCACGTCGTGGTCTCGGTCGGCCGCTCCGCCGAGCTGCTCGTCGGGCTG  26040
       T  P  G  R  H  V  V  V  S  V  G  R  S  A  E  L  L  V  G  L
                .         .         .         .         .         .
26041  CTCGGCGTGCTCAAGGCGGGTGGCGCCTTCGTCCCCGTCGACGTGGGCTTCCCCCGCAAA  26100
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       L  G  V  L  K  A  G  G  A  F  V  P  V  D  V  G  F  P  R  K
                .         .         .         .         .         .
26101  CGGCTGGAGTTCGTGCTCCGGGAGACCGCCGCGCCGGTCCTGCTCTGCACCGCCGACGTA  26160
       R  L  E  F  V  L  R  E  T  A  A  P  V  L  L  C  T  A  D  V
                .         .         .         .         .         .
26161  CGGGACCGCATCGGCACTCGGACCCTCGACGACGCCGGGGTGACACCCGTCGCGCTGGAC  26220
       R  D  R  I  G  T  R  T  L  D  D  A  G  V  T  P  V  A  L  D
                .         .         .         .         .         .
26221  GCCGACCGGCGGCGCATCGCCGCACACCCCGCCGGCCCCACCGGCATCGCCACCACCCCC  26280
       A  D  R  R  R  I  A  A  H  P  A  G  P  T  G  I  A  T  T  P
                .         .         .         .         .         .
26281  GACGCCCCCGCGTACGTCGTCTACACCTCCGGCACCACCGGGAAGCCCAACGGCGTACGC  26340
       D  A  P  A  Y  V  V  Y  T  S  G  T  T  G  K  P  N  G  V  R
                .         .         .         .         .         .
26341  GTCCCGCACCGGGGCCTCACCAACTACCTCACCTGGTGCACCGGCGCCTACGGACTCGAC  26400
       V  P  H  R  G  L  T  N  Y  L  T  W  C  T  G  A  Y  G  L  D
                .         .         .         .         .         .
26401  GGGGGCACCGGCACCCTCGTGCACACCTCCATCAGCTTCGACCTCACCCTCACCACCCTG  26460
       G  G  T  G  T  L  V  H  T  S  I  S  F  D  L  T  L  T  T  L
                .         .         .         .         .         .
26461  TTCGGCCCCCTGCTCGCCGGCGGGCAGGTGGTCATGCTCTCCGAGACCGCCGGCGTGACC  26520
       F  G  P  L  L  A  G  G  Q  V  V  M  L  S  E  T  A  G  V  T
                .         .         .         .         .         .
26521  GGCCTGATCGCCGCGCTGCGCTCCCGGCGCGACCTCACCCTGGTCAAGCTGACCCCGACC  26580
       G  L  I  A  A  L  R  S  R  R  D  L  T  L  V  K  L  T  P  T
                .         .         .         .         .         .
26581  CACCTCGACGTCGTCAACCAGCTGCTCACCCCCGACGAGCTGCGCGGCGCGGTCCGCACC  26640
       H  L  D  V  V  N  Q  L  L  T  P  D  E  L  R  G  A  V  R  T
                .         .         .         .         .         .
26641  CTCGTCGTCGGCGGGGAGGCGGTGCGGGCGGAGAGCCTGGAGCCGTTCCGGGCCTCCGGG  26700
       L  V  V  G  G  E  A  V  R  A  E  S  L  E  P  F  R  A  S  G
                .         .         .         .         .         .
26701  ACGCGGGTCGTCAACGAGTACGGGCCCAGCGAGACGGTCGTCGGCAGCGTCGCGCACGTC  26760
       T  R  V  V  N  E  Y  G  P  S  E  T  V  V  G  S  V  A  H  V
                .         .         .         .         .         .
26761  GTCGACGCCGCCACGCCCCGTACCGGCCCGGTGCCCATCGGCCGGCCGATCGCCAACACC  26820
       V  D  A  A  T  P  R  T  G  P  V  P  I  G  R  P  I  A  N  T
                .         .         .         .         .         .
26821  ACCGTCCACCTGCTCGACCAGCGGCGGCGGCCCGTCCCCGACGGCGTCGTCGGCGAGCTG  26880
       T  V  H  L  L  D  Q  R  R  R  P  V  P  D  G  V  V  G  E  L
                .         .         .         .         .         .
26881  TGGATCGGCGGCGCCGGTGTCGCCGACGGCTACCTGGGGCGGCCGGAACTCACCGGCGAG  26940
       W  I  G  G  A  G  V  A  D  G  Y  L  G  R  P  E  L  T  G  E
                .         .         .         .         .         .
26941  CGCTTCCTCCCCAGCGACTACCCGCCGGACGGCGGCCGGGTCTACCGCACCGGCGACCTG  27000
       R  F  L  P  S  D  Y  P  P  D  G  G  R  V  Y  R  T  G  D  L
                .         .         .         .         .         .
27001  GCCCGCCGGCGCGCCGACGGCACCCTGGAGTACCTCGGGCGCACCGACGCGCAGGTGAAG  27060
       A  R  R  R  A  D  G  T  L  E  Y  L  G  R  T  D  A  Q  V  K
                .         .         .         .         .         .
27061  ATCCGCGGCGTCCGGGTGGAGCCCGCCGAGACCGAGGCCGTCCTCGCCTCCCACCCCGGC  27120
       I  R  G  V  R  V  E  P  A  E  T  E  A  V  L  A  S  H  P  G
                .         .         .         .         .         .
27121  GTCGGCCAGGCCGTCGTGGTCGCCCGGCTGGACGAGGACCCCGGCCGTTCGTCGCCGCTC  27180
       V  G  Q  A  V  V  V  A  R  L  D  E  D  P  G  R  S  S  P  L
                .         .         .         .         .         .
27181  GCCGGCGAGCTGACGCTGACCGGCTACGTGGTCCCGGCCCGCGGTGCCCAGGCGCCCCCG  27240
       A  G  E  L  T  L  T  G  Y  V  V  P  A  R  G  A  Q  A  P  P
                .         .         .         .         .         .
27241  CACGAGGAGCTCATCGCGTACTGCCGGGAGCGGCTGCCCGAGCACTTCGTCCCGGCCGTC  27300
       H  E  E  L  I  A  Y  C  R  E  R  L  P  E  H  F  V  P  A  V
                .         .         .         .         .         .
27301  CTCGTCACCCTCGACGCCCTGCCCGTCACCGGCCACGGCAAGATCGACCGCGGTGCGCTG  27360
       L  V  T  L  D  A  L  P  V  T  G  H  G  K  I  D  R  G  A  L
                .         .         .         .         .         .
27361  CCCAAGCCGCACGCCCGGGCCCGGGACGGCGCGGCGTACGTCGCGCCGCGCACCGCCACC  27420
       P  K  P  H  A  R  A  R  D  G  A  A  Y  V  A  P  R  T  A  T
                .         .         .         .         .         .
27421  GAGGAGATCCTCGCGGCCACCGTCGCGAAGGTGCTGGGCGTCGAGCGCGTCGGCATCGAC  27480
       E  E  I  L  A  A  T  V  A  K  V  L  G  V  E  R  V  G  I  D
                .         .         .         .         .         .
27481  GACAACTACTTCGTCCTGGGCGGCGACTCCATCCGCAGCGTCATGGTCGCCAGCCGGGCC  27540
       D  N  Y  F  V  L  G  G  D  S  I  R  S  V  M  V  A  S  R  A
                .         .         .         .         .         .
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27541  CAGGCCCGCGGGGTCGAGGTCACCGTGGCCGACCTGCACCGGCACCCCACCGTCCGGGCC  27600
       Q  A  R  G  V  E  V  T  V  A  D  L  H  R  H  P  T  V  R  A
                .         .         .         .         .         .
27601  TGCGCCGCGCACCTGGACGCCCGCGAGGACCTGCCGCGGACGCCCGTCACCGAACCCTTC  27660
       C  A  A  H  L  D  A  R  E  D  L  P  R  T  P  V  T  E  P  F
                .         .         .         .         .         .
27661  GCGCTGATCTCCGCCGAGGACCGGGCGCTGGTGCCGGACGACGTCGAGGACGCCTTCCCG  27720
       A  L  I  S  A  E  D  R  A  L  V  P  D  D  V  E  D  A  F  P
                .         .         .         .         .         .
27721  CTGAACCTGCTCCAGGAAGGCATGATCTTCCACCGCGACTTCGCGGCGAAGTCGGCCGTC  27780
       L  N  L  L  Q  E  G  M  I  F  H  R  D  F  A  A  K  S  A  V
                .         .         .         .         .         .
27781  TACCACGCCATCGCGTCCGTGCGGCTGCGCGCCCCGTTCGACCTCGCCGTGCTGCGGATG  27840
       Y  H  A  I  A  S  V  R  L  R  A  P  F  D  L  A  V  L  R  M
                .         .         .         .         .         .
27841  GTCGTGCGCCAGCTCGTCGAGCGGCACCCGATGCTGCGCACCTCCTTCGACATGAGCCGC  27900
       V  V  R  Q  L  V  E  R  H  P  M  L  R  T  S  F  D  M  S  R
                .         .         .         .         .         .
27901  TTCAGCCGCCCGCTGCAACTGGTGCACCGCGAGTTCGCCGATCCGCTGCACTACGAGGAC  27960
       F  S  R  P  L  Q  L  V  H  R  E  F  A  D  P  L  H  Y  E  D
                .         .         .         .         .         .
27961  CTGCGCGGCAGGAGCGCCGAGGAGCAGGACGCCCGCGTCGAGGAGTGGATCGAGCGGGAG  28020
       L  R  G  R  S  A  E  E  Q  D  A  R  V  E  E  W  I  E  R  E
                .         .         .         .         .         .
28021  AAGGAACGCGGCTTCGAGCTGCACGAGTTCCCGCTGATCCGCTTCATGGCGCAGCGCCTG  28080
       K  E  R  G  F  E  L  H  E  F  P  L  I  R  F  M  A  Q  R  L
                .         .         .         .         .         .
28081  GAGGACGACGTCTTCCAGTTCACCTACGGCTTCCACCACGAGATCGTGGACGGCTGGAGC  28140
       E  D  D  V  F  Q  F  T  Y  G  F  H  H  E  I  V  D  G  W  S
                .         .         .         .         .         .
28141  GAAGCCCTGATGATCACCGAGCTGTTCAGCCACTACTTCTCGGTGATCTACGACGAGCCG  28200
       E  A  L  M  I  T  E  L  F  S  H  Y  F  S  V  I  Y  D  E  P
                .         .         .         .         .         .
28201  ATCGCGATCAAGCCACCCACCGCCGGCATGCGCGACGCCGTCGCCCTGGAGCTGGAGGCC  28260
       I  A  I  K  P  P  T  A  G  M  R  D  A  V  A  L  E  L  E  A
                .         .         .         .         .         .
28261  CTCGCGGACCGCCGCAACTACGAGTTCTGGGACTCCTACCTCGCCGACGCCACCCTGATG  28320
       L  A  D  R  R  N  Y  E  F  W  D  S  Y  L  A  D  A  T  L  M
                .         .         .         .         .         .
28321  CGGCTGCCCAGGCCCGGCACCGGACCCCGGGCCGACAAGGGCGACCGGGACATCACCCGC  28380
       R  L  P  R  P  G  T  G  P  R  A  D  K  G  D  R  D  I  T  R
                .         .         .         .         .         .
28381  ATCGCCGTCCCCGTCCCCACCGAACTCTCCGACGGCCTCAAGCGGGTCGCCGCCACCCAC  28440
       I  A  V  P  V  P  T  E  L  S  D  G  L  K  R  V  A  A  T  H
                .         .         .         .         .         .
28441  GCCGTCCCGCTGAAGACCGTGCTCCTGGCCGCGCACATGGTGGTGATGTCCCTCTACGGC  28500
       A  V  P  L  K  T  V  L  L  A  A  H  M  V  V  M  S  L  Y  G
                .         .         .         .         .         .
28501  GGCCACGAGGACACCCTCACCTACACCGTCACCAACGGCCGCCCCGAGACCGCCGACGGC  28560
       G  H  E  D  T  L  T  Y  T  V  T  N  G  R  P  E  T  A  D  G
                .         .         .         .         .         .
28561  AGCACCGCGATCGGGCTGTTCGTCAACAGCCTCGCGCTCCGCGTCCGGATGACCGGCGGC  28620
       S  T  A  I  G  L  F  V  N  S  L  A  L  R  V  R  M  T  G  G
                .         .         .         .         .         .
28621  ACCTGGGCCGACCTGATCACCGCCACGCTGGAGTCCGAGCGCGCCTCGATGCCGTACCGG  28680
       T  W  A  D  L  I  T  A  T  L  E  S  E  R  A  S  M  P  Y  R
                .         .         .         .         .         .
28681  CGGCTGCCGATGGCCGAACTCAAGCGCCACCAGGGCAACGAACCCCTGGCCGAGACGCTG  28740
       R  L  P  M  A  E  L  K  R  H  Q  G  N  E  P  L  A  E  T  L
                .         .         .         .         .         .
28741  TTCTTCTTCACCAACTACCACGTCTTCCACGTGCTCGACCGCTGGATCGACCGCGGCGTC  28800
       F  F  F  T  N  Y  H  V  F  H  V  L  D  R  W  I  D  R  G  V
                .         .         .         .         .         .
28801  GGCCACGTCGCCAACGAGCTCTACGGCGAGTCCACCTTCCCCTTCTGCGGCATCTTCCGC  28860
       G  H  V  A  N  E  L  Y  G  E  S  T  F  P  F  C  G  I  F  R
                .         .         .         .         .         .
28861  CTGAACCGGGAGACCGGCGAGCTGGAGGTCCGCATCGAGTACGACAGCCTGCAGTTCTCC  28920
       L  N  R  E  T  G  E  L  E  V  R  I  E  Y  D  S  L  Q  F  S
                .         .         .         .         .         .
28921  GACGCCCTCATGGAGAGCGTCCGCGACAGCTACGCCCGCGTCCTCGCGGCCCTGGTCGCC  28980
       D  A  L  M  E  S  V  R  D  S  Y  A  R  V  L  A  A  L  V  A
                .         .         .         .         .         .
28981  GACCCCGACGGGCGCTACGACCGGCACGAGTTCCGCTCCGACCGCGACCGGGCCGCACTG  29040
       D  P  D  G  R  Y  D  R  H  E  F  R  S  D  R  D  R  A  A  L
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                 .         .         .         .         .         .
29041   GCCGTCCTCACCCGCGGGCCCGAGGCGCCGGCGGCCGACCGGTGCCTGCACGACCTGGTG   29100
        A  V  L  T  R  G  P  E  A  P  A  A  D  R  C  L  H  D  L  V
                 .         .         .         .         .         .
29101   GCGGACCGGGCGGCGGACCGCCCCGACGCCCCGGCCGTCCAGCTGGACACCGACGTGCTC   29160
        A  D  R  A  A  D  R  P  D  A  P  A  V  Q  L  D  T  D  V  L
                 .         .         .         .         .         .
29161   AGCTACGGCGAGCTCGACCGCCGCGCCAACCGGCTGGCCCACCACCTGCGTTCGCTCGGC   29220
        S  Y  G  E  L  D  R  R  A  N  R  L  A  H  H  L  R  S  L  G
                 .         .         .         .         .         .
29221   ATCGGCCCGGAGAGCGTCGTCGGCGTCCTGGCCGAACGCTCCCTCGCCCAGATCATCGGC   29280
        I  G  P  E  S  V  V  G  V  L  A  E  R  S  L  A  Q  I  I  G
                 .         .         .         .         .         .
29281   CTCCTCGCGGTCCTCAAGGCGGGCGCCGCCTACGTCCCGCTCGACCCGGCCCAGCCCGAC   29340
        L  L  A  V  L  K  A  G  A  A  Y  V  P  L  D  P  A  Q  P  D
                 .         .         .         .         .         .
29341   GAGCGCCTCGCCGCCGTCATCGCCGGGAGCGGGGCCGCCGCCGTCCTCCACCGGCCCGGC   29400
        E  R  L  A  A  V  I  A  G  S  G  A  A  A  V  L  H  R  P  G
                 .         .         .         .         .         .
29401   CTCGAAGGGCGGCTGCCCGCGGGCGTCCGCGCGCTCCCCACCGACGCCGCCGACGGCAGC   29460
        L  E  G  R  L  P  A  G  V  R  A  L  P  T  D  A  A  D  G  S
                 .         .         .         .         .         .
29461   ACCGCCACGCACGACCCCGGGCCCACCGCCACGCCCCGCAACGCCGCGTACGTGATGTAC   29520
        T  A  T  H  D  P  G  P  T  A  T  P  R  N  A  A  Y  V  M  Y
                 .         .         .         .         .         .
29521   ACCTCCGGATCCACCGGAGAGCCCAAGGGCATCGTCGTCGAACACCGCAACGTCGTGGCC   29580
        T  S  G  S  T  G  E  P  K  G  I  V  V  E  H  R  N  V  V  A
                 .         .         .         .         .         .
29581   TCCCTCGCCGCCCGCGGCGCCCACTACGCGGCCGGACCCGGCCGGTTCCTGCTGCTGTCC   29640
        S  L  A  A  R  G  A  H  Y  A  A  G  P  G  R  F  L  L  L  S
                 .         .         .         .         .         .
29641   TCCTTCGCCTTCGACAGCTCGGTCGCCGGCATCTTCTGGACGCTGACCCAGGGCGGCACC   29700
        S  F  A  F  D  S  S  V  A  G  I  F  W  T  L  T  Q  G  G  T
                 .         .         .         .         .         .
29701   CTCGTCCTGCCCGGCGAGGGACAGCAACTCGACCCCGCCGCGCTGGTGGAGACCATCGCC   29760
        L  V  L  P  G  E  G  Q  Q  L  D  P  A  A  L  V  E  T  I  A
                 .         .         .         .         .         .
29761   CGGCAACGGCCCACCCACACCCTCGCCATCCCCTCCCTGCTGGCGCCCGTCCTGGACCAG   29820
        R  Q  R  P  T  H  T  L  A  I  P  S  L  L  A  P  V  L  D  Q
                 .         .         .         .         .         .
29821   GCCGCCCCCGGCGACCTCGCCTCCCTGCGCACGGTGATCGCCGCGGGCGAGTCCTGTCCG   29880
        A  A  P  G  D  L  A  S  L  R  T  V  I  A  A  G  E  S  C  P
                 .         .         .         .         .         .
29881   GCCGAACTGGCCGCCGCCTGCCGGGACCTGCTGCCCGGGAGCACCTTCCACAACGAGTAC   29940
        A  E  L  A  A  A  C  R  D  L  L  P  G  S  T  F  H  N  E  Y
                 .         .         .         .         .         .
29941   GGCCCCACCGAGACCACCGTGTGGAGCACCGTCTGGTCCCAGGAGAACGAGCACGACGGA   30000
        G  P  T  E  T  T  V  W  S  T  V  W  S  Q  E  N  E  H  D  G
                 .         .         .         .         .         .
30001   CCCCACCTCCCCATCGGCCGGCCGGTCGCGGGCACCTGGGTGCACCCCCGCGACCACCGC   30060
        P  H  L  P  I  G  R  P  V  A  G  T  W  V  H  P  R  D  H  R
                 .         .         .         .         .         .
30061   GGACGCACCGTCCCCCTCGGCGTCGCCGGCGAACTCTCCATCGGCGGCGCCGGCGTGGCC   30120
        G  R  T  V  P  L  G  V  A  G  E  L  S  I  G  G  A  G  V  A
                 .         .         .         .         .         .
30121   CGCGGCTACCTCGGGCGCCCCCGGGACACCGCGGCCGCCTTCCGCCCCGACCCCGAGGCC   30180
        R  G  Y  L  G  R  P  R  D  T  A  A  A  F  R  P  D  P  E  A
                 .         .         .         .         .         .
30181   ACGGCTCCCGGCGGCCGCGCCTACGCCACCGGCGACCTCGGCCGCTACCTCCCCGACGGC   30240
        T  A  P  G  G  R  A  Y  A  T  G  D  L  G  R  Y  L  P  D  G
                 .         .         .         .         .         .
30241   AACCTGGAGTTCCTCGGCCGCGCCGACCACCAGGTCAAGATCCGCGGCTTCCGGGTCGAG   30300
        N  L  E  F  L  G  R  A  D  H  Q  V  K  I  R  G  F  R  V  E
                 .         .         .         .         .         .
30301   CTCGGCGAGATCGAGGCCGTCCTCGACACCCACCCGGAGCTCCAGCGGACCATCGTCATG   30360
        L  G  E  I  E  A  V  L  D  T  H  P  E  L  Q  R  T  I  V  M
                 .         .         .         .         .         .
30361   GCACGCGGCGACCACCCCGGCGACCAGGTGCTCGTCGCCTACGTCCTCCCCGCCCCCGGC   30420
        A  R  G  D  H  P  G  D  Q  V  L  V  A  Y  V  L  P  A  P  G
                 .         .         .         .         .         .
30421   CGGCGGCCCGAACCCGCCGACATCCAGGGGTACGTCCGCGACCGGCTGCCCCGCTACATG   30480
        R  R  P  E  P  A  D  I  Q  G  Y  V  R  D  R  L  P  R  Y  M
                 .         .         .         .         .         .
30481   GTGCCCACCGCGGTGATCGTCCTCGACGCGGTACCGCTGACCGCCGCCGGCAAGGTCGAC   30540
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        V  P  T  A  V  I  V  L  D  A  V  P  L  T  A  A  G  K  V  D
                 .         .         .         .         .         .
30541   CGGGCCTCGCTCCCCGCCCCCAGCCACGCCCAGCTCACCCGGGACCAGGAGTACGTCGAG   30600
        R  A  S  L  P  A  P  S  H  A  Q  L  T  R  D  Q  E  Y  V  E
                 .         .         .         .         .         .
30601   CCCGGCACCGACACCGAGCGGGCGCTCGCCGCCATCTGGGCCGACGTCCTCAAACTGGAC   30660
        P  G  T  D  T  E  R  A  L  A  A  I  W  A  D  V  L  K  L  D
                 .         .         .         .         .         .
30661   CGGATCGGGGCCGGTGACCGCTTCTTCGACGTCGGCGGCGAATCCCTGCGCGCGATGCAG   30720
        R  I  G  A  G  D  R  F  F  D  V  G  G  E  S  L  R  A  M  Q
                 .         .         .         .         .         .
30721   GCCACCGCCGCGGCCAACAAGATGTTCCGCACCCGCGTCTCCGTCCGCCGCCTCTTCGAG   30780
        A  T  A  A  A  N  K  M  F  R  T  R  V  S  V  R  R  L  F  E
                 .         .         .         .         .         .
30781   GCGCCCTCCCTGCGGGAGTTCGCCCACGAGATCGACAAGGCCCGCCTCGCGGGCGGCGGG   30840
        A  P  S  L  R  E  F  A  H  E  I  D  K  A  R  L  A  G  G  G
                 .         .         .         .         .         .
30841   ACCGGCCTCACCGGCCCCGCGGCCGCCCCGGCCACCGGAGGTGCCGCCGAATGACCCCGG   30900
        T  G  L  T  G  P  A  A  A  P  A  T  G  G  A  A  E  *
                                                          M  T  P  A    (orf25)
                 .         .         .         .         .         .
30901   CCGCCGACACCACCCACCCGCTCTCGCCGGCCCAGCGCAGCATGTGGTTCCTGCACCGGC   30960
          A  D  T  T  H  P  L  S  P  A  Q  R  S  M  W  F  L  H  R  L
                 .         .         .         .         .         .
30961   TCGCGCCCGAGGTGCCCGCCTACAACATCTGCACCGCCATCGAGCTCACCGGCACACCGC   31020
          A  P  E  V  P  A  Y  N  I  C  T  A  I  E  L  T  G  T  P  R
                 .         .         .         .         .         .
31021   GCCCGGCGGCGCTGCGGGACGTGGTACGGCGGCTCGGCCGCAGGCACGAGGCGCTGCGCA   31080
          P  A  A  L  R  D  V  V  R  R  L  G  R  R  H  E  A  L  R  T
                 .         .         .         .         .         .
31081   CGGTGTTCCCGTCGGTGGGGGAGACCCCCCGCCAACGGGTCACCGACCGGGCGGCGCCCC   31140
          V  F  P  S  V  G  E  T  P  R  Q  R  V  T  D  R  A  A  P  L
                 .         .         .         .         .         .
31141   TGCGGACCGTGGACCTCACCCACCTGACCCCCGCCGCCGCCGAGGCCGAGACCGCACGGA   31200
          R  T  V  D  L  T  H  L  T  P  A  A  A  E  A  E  T  A  R  T
                 .         .         .         .         .         .
31201   CGCTACGGTGCGCCGCCGCCCGGCCGTTCCGGCTCGACACCGGCCCCCTGGCGGAATGGA   31260
          L  R  C  A  A  A  R  P  F  R  L  D  T  G  P  L  A  E  W  T
                 .         .         .         .         .         .
31261   CCCTGCTGCGCCGCGCCCCCGGCCACGCGCTGCTCGTCCTCTCCGTCCACCACATCGTCT   31320
          L  L  R  R  A  P  G  H  A  L  L  V  L  S  V  H  H  I  V  F
                 .         .         .         .         .         .
31321   TCGACGGCGGCTCGCTCCACGTGGTCTGCCGCGAACTGGAGGAGGCGTACGGAGCGGCCC   31380
          D  G  G  S  L  H  V  V  C  R  E  L  E  E  A  Y  G  A  A  L
                 .         .         .         .         .         .
31381   TCGCCGGGCGCCCGGACCCCCTCGGCACACCCGCGCCGGGCTACGGACGGCAGTGCCGGA   31440
          A  G  R  P  D  P  L  G  T  P  A  P  G  Y  G  R  Q  C  R  T
                 .         .         .         .         .         .
31441   CGCGGGCGGCGGAACAGGACGAGGCCGGGCGGGAGTTCTGGCGCCGCGAACTGTCCGGCG   31500
          R  A  A  E  Q  D  E  A  G  R  E  F  W  R  R  E  L  S  G  A
                 .         .         .         .         .         .
31501   CGCCACCCCGCACGACCGTCTTCCGGGGCACCGGCCGGCCCGCCGGACCGCCCGCCCGCG   31560
          P  P  R  T  T  V  F  R  G  T  G  R  P  A  G  P  P  A  R  A
                 .         .         .         .         .         .
31561   CCACCGTCCACTACGGCACCGACGATCCGGCCCCGACCGCGGACTTCTGCCGCGAGCACG   31620
          T  V  H  Y  G  T  D  D  P  A  P  T  A  D  F  C  R  E  H  A
                 .         .         .         .         .         .
31621   CCGTCACCGGCTACGTGCTGCTGCTCGCGGCCCTCGCCTGCCTGGTCGCCCGGTACACCG   31680
          V  T  G  Y  V  L  L  L  A  A  L  A  C  L  V  A  R  Y  T  G
                 .         .         .         .         .         .
31681   GCCGGACGGACGTGGTGATCGGCTCACCCGTCGGACTGCGCGAGGACCCCGAAGGGCTCG   31740
          R  T  D  V  V  I  G  S  P  V  G  L  R  E  D  P  E  G  L  A
                 .         .         .         .         .         .
31741   CCACCGTCGGCCCGATGCTCAACCTGCTGCCGCTGCGCCTCCGGCTGCACGGCGACCCCG   31800
          T  V  G  P  M  L  N  L  L  P  L  R  L  R  L  H  G  D  P  G
                 .         .         .         .         .         .
31801   GCTTCGGCGAGGTCCTGGCCCGCACCCGGGAGACGCTGCTCGGCGCGCTGGAGCACCGCA   31860
          F  G  E  V  L  A  R  T  R  E  T  L  L  G  A  L  E  H  R  T
                 .         .         .         .         .         .
31861   CCACACCGTTCGAGGACATCGTCGACGCGGTGGGCGCCGACCGGGACCCGGACGTCAGCC   31920
          T  P  F  E  D  I  V  D  A  V  G  A  D  R  D  P  D  V  S  P
                 .         .         .         .         .         .
31921   CCCTCTTCCAGATCCTCTTCGCCCACGAACGCCCCCCGGCCCCACCCGCGTTACCGGGCG   31980
          L  F  Q  I  L  F  A  H  E  R  P  P  A  P  P  A  L  P  G  V
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                .         .         .         .         .         .
31981  TCCGTGCCCGCGTCGTACCCGTCCCCGCTCCGGCCGCCAAGTACGAGCTCGCCGTCACCG   32040
         R  A  R  V  V  P  V  P  A  P  A  A  K  Y  E  L  A  V  T  A
                .         .         .         .         .         .
32041  CCACCGAGACGCCCGACGGGCTCCGGCTGATCGTCGAGGCGGAGCACGGACACGGGGAAC   32100
         T  E  T  P  D  G  L  R  L  I  V  E  A  E  H  G  H  G  E  P
                .         .         .         .         .         .
32101  CGGCCGAACTCGCCGCCTTCGCCCGCCACTTCGGCGTCCTGCTGGCCGCCGGGGTCCGCG   32160
         A  E  L  A  A  F  A  R  H  F  G  V  L  L  A  A  G  V  R  A
                .         .         .         .         .         .
32161  CGCCGGACACACCGCTGAGCCGCCTGCCGCTGCTCACCGACGAGGAGCGGCGCCGGCTCA   32220
         P  D  T  P  L  S  R  L  P  L  L  T  D  E  E  R  R  R  L  T
                .         .         .         .         .         .
32221  CCGACACCACGGCCCCCCGCACCGCGCCGGAGGCCCCCTACCGCCCCCTGCACCGGCTGG   32280
         D  T  T  A  P  R  T  A  P  E  A  P  Y  R  P  L  H  R  L  V
                .         .         .         .         .         .
32281  TCGAGGAGTCCGCCGCCCGCCGGCCCGACGCCCTGGCGGTCGTCGGCGGCACGCGTCACC   32340
         E  E  S  A  A  R  R  P  D  A  L  A  V  V  G  G  T  R  H  L
                .         .         .         .         .         .
32341  TCAGCTACCGGGAGCTGAACTGCCGCGCCAACCGGCGTGCCGCCTGGCTGCGCCGCGCTG   32400
         S  Y  R  E  L  N  C  R  A  N  R  R  A  A  W  L  R  R  A  G
                .         .         .         .         .         .
32401  GCATCGGCACCGAGGACGTGGTCGGCGTCCGGCTGGAACGCGGCCCGGAACTCCTCGTCT   32460
         I  G  T  E  D  V  V  G  V  R  L  E  R  G  P  E  L  L  V  S
                .         .         .         .         .         .
32461  CGCTCCTCGCCGTCCTCAAGGCCGGCGCCGCCTACCTGCCCGTCGACCCGGCGCTGCCCG   32520
         L  L  A  V  L  K  A  G  A  A  Y  L  P  V  D  P  A  L  P  A
                .         .         .         .         .         .
32521  CCGAGCGGGTACGGCTGATGCTCGACGACGCCCGGGCCGCGCTGCTGCTCACCGAGACCG   32580
         E  R  V  R  L  M  L  D  D  A  R  A  A  L  L  L  T  E  T  A
                .         .         .         .         .         .
32581  CGCTCGGCACCCCGCCGGCCCCGGCCGGCACCCCCGTGCACCACGTGGACGGACCGCCAC   32640
         L  G  T  P  P  A  P  A  G  T  P  V  H  H  V  D  G  P  P  P
                .         .         .         .         .         .
32641  CGCCGACCCGGCCCGGGGACGACGCCGACCACACCGGCCCCGACCTGCCCACCAGCCTCG   32700
         P  T  R  P  G  D  D  A  D  H  T  G  P  D  L  P  T  S  L  A
                .         .         .         .         .         .
32701  CCTACCTCCTCTACACCTCCGGGTCGACGGGCCGGCCCAAGGCCGTGGCCCTCCAGCACG   32760
         Y  L  L  Y  T  S  G  S  T  G  R  P  K  A  V  A  L  Q  H  D
                .         .         .         .         .         .
32761  ACAGCGCCGCGGCGTTCCTGCGCTGGGCGGGCCGCGCCTTCGACGGCGGGGAGCTGGCCG   32820
         S  A  A  A  F  L  R  W  A  G  R  A  F  D  G  G  E  L  A  A
                .         .         .         .         .         .
32821  CCGTCCTGGCCACCACCTCCGCCGGCTTCGACCTGTCGGTCTTCGAGCTGTTCGCCCCCC   32880
         V  L  A  T  T  S  A  G  F  D  L  S  V  F  E  L  F  A  P  L
                .         .         .         .         .         .
32881  TGGCCCACGGCGGCACCGTCGTCCTCGCCGACAGCGCCCTGCACGTGCCCGCCCTGCCCT   32940
         A  H  G  G  T  V  V  L  A  D  S  A  L  H  V  P  A  L  P  W
                .         .         .         .         .         .
32941  GGGCGCCCGCGGCGACGCTCCTGAACACCGTGCCCTCCGCGGCCGCCGCCCTGCTGGACG   33000
         A  P  A  A  T  L  L  N  T  V  P  S  A  A  A  A  L  L  D  A
                .         .         .         .         .         .
33001  CCGACGGCCTGCCCGACGGTCTGACGGCCGTCAACCTGGCGGGCGAGCCCCTGACCGCGG   33060
         D  G  L  P  D  G  L  T  A  V  N  L  A  G  E  P  L  T  A  E
                .         .         .         .         .         .
33061  AGCTGGTCGCCCGGCTGCACGCCCGCCTGCCGAAGGCCGCCGTCCGCAACCTCTACGGCC   33120
         L  V  A  R  L  H  A  R  L  P  K  A  A  V  R  N  L  Y  G  P
                .         .         .         .         .         .
33121  CCTCGGAGGCCACCACCTACGCCACCGCGGCCCTCGTGCCCGCGGGCGGCACCGAGGCGC   33180
         S  E  A  T  T  Y  A  T  A  A  L  V  P  A  G  G  T  E  A  P
                .         .         .         .         .         .
33181  CGGCCATCGGCCGGGCGCTCGGCGCGGCCCGCGTGTGGACCGCCGACGACCGGCAGCGCC   33240
         A  I  G  R  A  L  G  A  A  R  V  W  T  A  D  D  R  Q  R  P
                .         .         .         .         .         .
33241  CCCTCCCCGGCGCGGTCGTCGGTGAACTCCTCATCGGCGGTACGGCCCCGGCCCGCGGCT   33300
         L  P  G  A  V  V  G  E  L  L  I  G  G  T  A  P  A  R  G  Y
                .         .         .         .         .         .
33301  ACCTCGGCCGGCCGGGACCGACCGCCGACGCCTTCCGGCCCGATCCGACGGGACCGCCCG   33360
         L  G  R  P  G  P  T  A  D  A  F  R  P  D  P  T  G  P  P  G
                .         .         .         .         .         .
33361  GCTCCCGGCTCTACCGCACCGGGGACCTGGCCGTACGCCGCCCCGACGGCCGGTTCGTGT   33420
         S  R  L  Y  R  T  G  D  L  A  V  R  R  P  D  G  R  F  V  F
                .         .         .         .         .         .
33421  TCCTCGGCCGCAAGGACGAGCAGATCAAACTCCGCGGGGTGCGCATCGAACCGGGCGAGG   33480
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         L  G  R  K  D  E  Q  I  K  L  R  G  V  R  I  E  P  G  E  V
                 .         .         .         .         .         .
33481   TGGAAGCCGCTCTCCGCCAGTGCGCGCCGGTCGCCGCGGCCGCCGTCGTGCTCGCCGGGA   33540
         E  A  A  L  R  Q  C  A  P  V  A  A  A  A  V  V  L  A  G  T
                 .         .         .         .         .         .
33541   CCACCGCGGAGAACCACCGCCTCGTCGGCTTCGTCACCCCTTCGCCCGGCGCCCGCGTCG   33600
         T  A  E  N  H  R  L  V  G  F  V  T  P  S  P  G  A  R  V  D
                 .         .         .         .         .         .
33601   ACCCCGAGCGCACCCTCGCCGCGCTGCGTTCGCGCCTGCCCGCCGCCCTCGTGCCCGCCG   33660
         P  E  R  T  L  A  A  L  R  S  R  L  P  A  A  L  V  P  A  A
                 .         .         .         .         .         .
33661   CGCTGGTGGTGTGCGACGCCCTGCCGCTGACCGCCAACGGGAAGACCGACCGGGCCGCCC   33720
         L  V  V  C  D  A  L  P  L  T  A  N  G  K  T  D  R  A  A  L
                 .         .         .         .         .         .
33721   TCGCCCGGCGGGCGCGCGGACACCGGCCGGACCACGGCGCGTACGCCCCGCCCCGCACCC   33780
         A  R  R  A  R  G  H  R  P  D  H  G  A  Y  A  P  P  R  T  R
                 .         .         .         .         .         .
33781   GCGTCGAGAAGGCGGTCGCCGCGATCTGGCGCGAGGTGCTCGGGACCGAACGGGTGGGGA   33840
         V  E  K  A  V  A  A  I  W  R  E  V  L  G  T  E  R  V  G  I
                 .         .         .         .         .         .
33841   TCCACCAGGGGTTCTTCGACGCGGGCGGCACCTCCCTGTCGCTGCTGCGCCTTCACCACC   33900
         H  Q  G  F  F  D  A  G  G  T  S  L  S  L  L  R  L  H  H  R
                 .         .         .         .         .         .
33901   GGCTGGTCGCGTCCGTCCATCCCGGCCTCCGGCTCGCCGACGTCTTCCGGCTGCCGACCG   33960
         L  V  A  S  V  H  P  G  L  R  L  A  D  V  F  R  L  P  T  V
                 .         .         .         .         .         .
33961   TCGCCGCGCTCGCCGCGTTCGTGGACGGGCAGGAGGACGCGCGCGAGACGGCCGTCGGCG   34020
         A  A  L  A  A  F  V  D  G  Q  E  D  A  R  E  T  A  V  G  D
                 .         .         .         .         .         .
34021   ACGCGGCCCTCCGGGCCGGCCGGCGCCGCGCCGCGGTGGCCGCGCGCCGCAGGAAAGGCG   34080
         A  A  L  R  A  G  R  R  R  A  A  V  A  A  R  R  R  K  G  G
                 .         .         .         .         .         .
34081   GCGGACGATGAGCCATGCCGACGCGGGCGACGGGCTCGACGCGGCTGACACGACTGACGC   34140
                M  S  H  A  D  A  G  D  G  L  D  A  A  D  T  T  D  A   (orf24)
         G  R  *
                 .         .         .         .         .         .
34141   GGCCGACGGGATCGCCGTGATCTCGCTGGGCGGACGCTTCCCCGGAGCGGACCGGGTGGA   34200
        A  D  G  I  A  V  I  S  L  G  G  R  F  P  G  A  D  R  V  D
                 .         .         .         .         .         .
34201   CCGCCTCTGGACGAACCTGCTCGACCGCGAGGACGCCATCAGCCACTTCACCGCCGACGA   34260
        R  L  W  T  N  L  L  D  R  E  D  A  I  S  H  F  T  A  D  E
                 .         .         .         .         .         .
34261   ACGCCTCGCCCGGGGCCGCGACCCCGAACTGGTGCGCCACCCGCGGTTCGTCGGCGCGGA   34320
        R  L  A  R  G  R  D  P  E  L  V  R  H  P  R  F  V  G  A  E
                 .         .         .         .         .         .
34321   AGGCGTCCTCGGCGACGTCTCCCTCTTCGACGCCGAGTTCTTCGGCTGCTCGCCGCGCGA   34380
        G  V  L  G  D  V  S  L  F  D  A  E  F  F  G  C  S  P  R  E
                 .         .         .         .         .         .
34381   GGCCGAAGTCATGGACCCGCAGCACCGGCTCTGCCTGGAGGAGGCGTGGCACGTCTTCGA   34440
        A  E  V  M  D  P  Q  H  R  L  C  L  E  E  A  W  H  V  F  D
                 .         .         .         .         .         .
34441   CACCGCCGGCTACGACCCGGCGGCGACGGGCACCGCGGTCGGGGTGTTCCTCTCCGCGAG   34500
        T  A  G  Y  D  P  A  A  T  G  T  A  V  G  V  F  L  S  A  S
                 .         .         .         .         .         .
34501   CCTCAGCTCGTACCTGATCCGCAACGTCCTGCCCGGCGGCGCGGCACAGCGCCTGCTCGG   34560
        L  S  S  Y  L  I  R  N  V  L  P  G  G  A  A  Q  R  L  L  G
                 .         .         .         .         .         .
34561   CGGCTTCCCGCTGCTGATCCACAACGACAAGGACTTTCTGGCCACCACCGTGTCCCACAA   34620
        G  F  P  L  L  I  H  N  D  K  D  F  L  A  T  T  V  S  H  K
                 .         .         .         .         .         .
34621   ACTGGGCCTCACCGGGCCGAGTTACGCCGTCGGCTCGGCCTGCTCGTCCTCCCTCGTCGC   34680
        L  G  L  T  G  P  S  Y  A  V  G  S  A  C  S  S  S  L  V  A
                 .         .         .         .         .         .
34681   GGTGCACCTGGCCTGCCAGAGCCTGCTCACCGAGGAATGCGACATGGCGCTGGCCGGCGG   34740
        V  H  L  A  C  Q  S  L  L  T  E  E  C  D  M  A  L  A  G  G
                 .         .         .         .         .         .
34741   GGTCTCGCTCCAAGTGCCGCAGGGCCAGGGGTACGTGCACGCCGACGACGGCATCTACTC   34800
        V  S  L  Q  V  P  Q  G  Q  G  Y  V  H  A  D  D  G  I  Y  S
                 .         .         .         .         .         .
34801   ACCCGACGGGCGCTGCGCCCCCTTCGACGCCGGCGCGGCGGGCACGGTGGGCGGCAGCGG   34860
        P  D  G  R  C  A  P  F  D  A  G  A  A  G  T  V  G  G  S  G
                 .         .         .         .         .         .
34861   CGTGGGCCTCGTCCTGCTCAAGCGGCTCGCCGACGCCGTGCGCGACGGGGACCGCGTCCA   34920
        V  G  L  V  L  L  K  R  L  A  D  A  V  R  D  G  D  R  V  H
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                .         .         .         .         .         .
34921  CGCGGTGATCCTCGGCTCGGCGGTGAACAACGACGGCGCCGACAAGGTCGGTTACACGGC   34980
        A  V  I  L  G  S  A  V  N  N  D  G  A  D  K  V  G  Y  T  A
                .         .         .         .         .         .
34981  GCCCGGCGTCACCGGCCAGAGCGCCGTCGTCGCCGAGGCCCTGGCGGTGGCCGGGATCTC   35040
        P  G  V  T  G  Q  S  A  V  V  A  E  A  L  A  V  A  G  I  S
                .         .         .         .         .         .
35041  CGCCGCGACCGTCGGCGTCCTGGAGGCGCACGGCACCGGCACCCGGCTGGGCGATCCCGT   35100
        A  A  T  V  G  V  L  E  A  H  G  T  G  T  R  L  G  D  P  V
                .         .         .         .         .         .
35101  CGAAGTGGCCGCGCTCACCCGGGCGTTCCGCGCCCACACGGACCGCAGCGGCTTCTGCGC   35160
        E  V  A  A  L  T  R  A  F  R  A  H  T  D  R  S  G  F  C  A
                .         .         .         .         .         .
35161  GCTGGGCTCGGTGAAGGCCAACGTGGGCCACCTGGACGCGGCGGCGGGCGTCACCGGGCT   35220
        L  G  S  V  K  A  N  V  G  H  L  D  A  A  A  G  V  T  G  L
                .         .         .         .         .         .
35221  GATCAAGGCCGTGCTGGCGGTCCGCGAGGGCGTCATCCCCGGCACCCCGCACTACCGTTC   35280
        I  K  A  V  L  A  V  R  E  G  V  I  P  G  T  P  H  Y  R  S
                .         .         .         .         .         .
35281  GCCCAACCCCGCCATCGACTTCGCCACCACACCCTTCTACGTCACCGCCGACACCCTCGC   35340
        P  N  P  A  I  D  F  A  T  T  P  F  Y  V  T  A  D  T  L  A
                .         .         .         .         .         .
35341  CTGGCCGGAGGCGGACCACCCCCGCCGGGCCGGCGTCAGCTCCTTCGGCATCGGGGGCAC   35400
        W  P  E  A  D  H  P  R  R  A  G  V  S  S  F  G  I  G  G  T
                .         .         .         .         .         .
35401  CAACGCCCACGTGATCCTGGAACAGGCCCCGCCGGCCGCCCCCGCGCGGACCGGACCGC   35460
        N  A  H  V  I  L  E  Q  A  P  P  A  A  P  R  A  D  R  T  A
                .         .         .         .         .         .
35461  CGGGGTGCCCATGCCGTTGGTGGTGTCCGCCCGCACCCGCGAAGCACTGGCGGAGGCCGT   35520
        G  V  P  M  P  L  V  V  S  A  R  T  R  E  A  L  A  E  A  V
                .         .         .         .         .         .
35521  CCGGGACCTGGCGGCGTGGTCGGCCCCGGAGCCGGGGACCCGGCTCGCCGATCTCGCCGC   35580
        R  D  L  A  A  W  S  A  P  E  P  G  T  R  L  A  D  L  A  A
                .         .         .         .         .         .
35581  CACGCTGGCCGGGCGCCGGGCCTTCCCGTACCGCGCCGCCGTCGTGTGCCACGACCTGCC   35640
        T  L  A  G  R  R  A  F  P  Y  R  A  A  V  V  C  H  D  L  P
                .         .         .         .         .         .
35641  CGAGGCCGCGCGCCTGCTGGGCGGCGCGCGCGGCGAGACCGCGCTCCCCGGCAGGGAGGC   35700
        E  A  A  R  L  L  G  G  A  R  G  E  T  A  L  P  G  R  E  A
                .         .         .         .         .         .
35701  CGTGTTCCTCTTCCCCGGGCAGGGCACCCTCCCGCCGGACACCGGGCGCGGCCTGTACGC   35760
        V  F  L  F  P  G  Q  G  T  L  P  P  D  T  G  R  G  L  Y  A
                .         .         .         .         .         .
35761  GGACGTGCCGGCGTTCCGCGCCCACTTCGACGCCTGTGCCGAAGGGTTCGCCCCGCTCGG   35820
        D  V  P  A  F  R  A  H  F  D  A  C  A  E  G  F  A  P  L  G
                .         .         .         .         .         .
35821  CACCGACCTCCACGCCGCGCTCGGGGCCCCGGCCGACGACACCAGGGCCGCGCAACCCGC   35880
        T  D  L  H  A  A  L  G  A  P  A  D  D  T  R  A  A  Q  P  A
                .         .         .         .         .         .
35881  CCTCTTCGCCGTCGAGTACGCCCTCGCCCGCACCCTGATGGACTGGGGTGTGCGCCCGGC   35940
        L  F  A  V  E  Y  A  L  A  R  T  L  M  D  W  G  V  R  P  A
                .         .         .         .         .         .
35941  CGCGATGCTCGGGCACAGCCTCGGCGAGTACGTCGCGGCGACGCTGGCCGGGGTGCTGTC   36000
        A  M  L  G  H  S  L  G  E  Y  V  A  A  T  L  A  G  V  L  S
                .         .         .         .         .         .
36001  CCTGCCGGACGCGCTGACGCTCGTCCGGGCCCGGGCGGAAGCGCAGCACACCATGCCGCC   36060
        L  P  D  A  L  T  L  V  R  A  R  A  E  A  Q  H  T  M  P  P
                .         .         .         .         .         .
36061  CGGCCGCATGCTCGCGGTCCCGCTCACGCCGGACGACCTGCGCCCGCTGCTGCCCCCGGA   36120
        G  R  M  L  A  V  P  L  T  P  D  D  L  R  P  L  L  P  P  E
                .         .         .         .         .         .
36121  GGTGGAGTTCAGCGCCTTCAACGCCCCCGGCCGCTGCGTCGTCGGCGGGCCCCCGGAGCC   36180
        V  E  F  S  A  F  N  A  P  G  R  C  V  V  G  G  P  P  E  P
                .         .         .         .         .         .
36181  GGTGGCGGAGCTGCGCGCCCGGCTGGCGCGGCGCGGAGTGCCGGCCGCCGAACTGGCCAC   36240
        V  A  E  L  R  A  R  L  A  R  R  G  V  P  A  A  E  L  A  T
                .         .         .         .         .         .
36241  CGCGCACGCCTTCCACTCGGCGGCCGTCGAACCGCTGCTGGACGGCTTCCGGGGCGTGCT   36300
        A  H  A  F  H  S  A  A  V  E  P  L  L  D  G  F  R  G  V  L
                .         .         .         .         .         .
36301  GGAAGGCGTCCGACTGCGGCCGCCCCGGCTGCGGTACGTGTCCTCCCTCACCGGCGACTG   36360
        E  G  V  R  L  R  P  P  R  L  R  Y  V  S  S  L  T  G  D  W
                .         .         .         .         .         .
36361  GGCCGACGCCGCGGTCACCACCCCCGCGTACTGGCTCGCCCACCTGCGCCGGCCCGTCCG   36420
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          A  D  A  A  V  T  T  P  A  Y  W  L  A  H  L  R  R  P  V  R
                  .         .         .         .         .         .
36421    CTTCGCCGACGGCCTGCGGCGCTGCCTGGACCTCGGCCCCGTCGCCCTGGTCGAGACCGG    36480
          F  A  D  G  L  R  R  C  L  D  L  G  P  V  A  L  V  E  T  G
                  .         .         .         .         .         .
36481    GCCGCGGGCCGGACTGACCGGCCTGGCCCGCCGCGCCGCGGGCCCCGGCGAGCCCCCTTA    36540
          P  R  A  G  L  T  G  L  A  R  R  A  A  G  P  G  E  P  P  Y
                  .         .         .         .         .         .
36541    CACCGTCCGCTGCCTGGCCGCCCCCGACGAGGCGGCTTCGCTGACCCACGCGGTCGCCGT    36600
          T  V  R  C  L  A  A  P  D  E  A  A  S  L  T  H  A  V  A  V
                  .         .         .         .         .         .
36601    ACTCTGGCGCTCGGGCTGCGCCGTCGACTGGACGGCGTTCCACCGCCCCGGGCGCCCCCG    36660
          L  W  R  S  G  C  A  V  D  W  T  A  F  H  R  P  G  R  P  R
                  .         .         .         .         .         .
36661    CCGCACCACCGTGCCCGGCTACCCCTTCCAACGGGTACGGCACTGGATCGACGCGCCGGA    36720
          R  T  T  V  P  G  Y  P  F  Q  R  V  R  H  W  I  D  A  P  D
                  .         .         .         .         .         .
36721    CGAGTCCGAACCCACGGACCTCGCCACCGCCCTGCGCGCGGAGTTGCGGACGGACGGCGA    36780
          E  S  E  P  T  D  L  A  T  A  L  R  A  E  L  R  T  D  G  D
                  .         .         .         .         .         .
36781    TCCGCCGCTCGCCGTCGATCAGCGGCCCGGACTGCGCACGGGGCTGAACCGGCTGTGCGC    36840
          P  P  L  A  V  D  Q  R  P  G  L  R  T  G  L  N  R  L  C  A
                  .         .         .         .         .         .
36841    CGCCCTGGCCCGCGACTACCTGGCCACCGGCGTCGAAGCGAGCGGGGTCCTGCCCGGATT    36900
          A  L  A  R  D  Y  L  A  T  G  V  E  A  S  G  V  L  P  G  F
                  .         .         .         .         .         .
36901    CCACCGCTTCCTGGACTACCTGCGCACCCTGGCCGCCTCCGCACCGGCCGCGGACGACGC    36960
          H  R  F  L  D  Y  L  R  T  L  A  A  S  A  P  A  A  D  D  A
                  .         .         .         .         .         .
36961    GGGGACGATCGCCGCGGAGATCACCGCGGCCCACCCGTCCTTCTCCGGGCTCGTCGACCT    37020
          G  T  I  A  A  E  I  T  A  A  H  P  S  F  S  G  L  V  D  L
                  .         .         .         .         .         .
37021    GCTCCGGCACTGCGCCCAGGGCTATCCGCGCGCCCTGTCCACCCCCGGAGCCGCACTGGA    37080
          L  R  H  C  A  Q  G  Y  P  R  A  L  S  T  P  G  A  A  L  D
                  .         .         .         .         .         .
37081    CGTCCTCTATCCGGCCGGCAGCGGCGACCTCCTGCGCCGCACCCTGGGCGAGGGCACCGC    37140
          V  L  Y  P  A  G  S  G  D  L  L  R  R  T  L  G  E  G  T  A
                  .         .         .         .         .         .
37141    CGACCACCGCGCCACCGGCCGCCTCACCCGGCTGGCCGGCTCCCTGCTCGACCGGCTCGC    37200
          D  H  R  A  T  G  R  L  T  R  L  A  G  S  L  L  D  R  L  A
                  .         .         .         .         .         .
37201    GGCCGACCGCGAACCCGGCCGCCCGCTGCGCGTCCTGGAGGCCGGAGCGGGCGCGGGCAG    37260
          A  D  R  E  P  G  R  P  L  R  V  L  E  A  G  A  G  A  G  S
                  .         .         .         .         .         .
37261    CCTCACCCAGGCCCTGGTCACCCGGGCCCCCGGCCGGCTCGACTACCACGCCACCGACAT    37320
          L  T  Q  A  L  V  T  R  A  P  G  R  L  D  Y  H  A  T  D  I
                  .         .         .         .         .         .
37321    CTCCCGGCACTTCGTGACCGCACTCGGCCGGGAGGCCGCCCGGCGCGGCCTGGACTTCGT    37380
          S  R  H  F  V  T  A  L  G  R  E  A  A  R  R  G  L  D  F  V
                  .         .         .         .         .         .
37381    CCGCGCACGCGTCCTCGACATCGCCCGCGACCCAGGCGAACAGGGCTTCGCCGGCGAGCG    37440
          R  A  R  V  L  D  I  A  R  D  P  G  E  Q  G  F  A  G  E  R
                  .         .         .         .         .         .
37441    GTTCGACGTCGTCTGCGGCCTCGACGTGGTCCACGCCACCCCCGACCTGCGCACCACGCT    37500
          F  D  V  V  C  G  L  D  V  V  H  A  T  P  D  L  R  T  T  L
                  .         .         .         .         .         .
37501    CGGCCATCTGCGCTCCCTGATGGCACCGGACGGCACCCTCGCGCTGATCGAGACCACCGC    37560
          G  H  L  R  S  L  M  A  P  D  G  T  L  A  L  I  E  T  T  A
                  .         .         .         .         .         .
37561    CGACGACCCCTGGCTGACGATGATCTGGGGCCTGACGGACGGCTGGTGGCACCACACCGA    37620
          D  D  P  W  L  T  M  I  W  G  L  T  D  G  W  W  H  H  T  D
                  .         .         .         .         .         .
37621    CCGGCGCACCCACGGCCCGCTGCTCGACGCCGCCGGCTGGCGCGCCCTCCTGGCCGGCGA    37680
          R  R  T  H  G  P  L  L  D  A  A  G  W  R  A  L  L  A  G  E
                  .         .         .         .         .         .
37681    GGACTTCGCCACGGCCGATGTGATCGTGCCGCCCGACGGCCCCCAGGACGCGGCCCTGCT    37740
          D  F  A  T  A  D  V  I  V  P  P  D  G  P  Q  D  A  A  L  L
                  .         .         .         .         .         .
37741    GCTCGCCCGGCAGACCCCCCGGCCGGCGGCGGCCGCACCGTCCGTCGGCAAGCGGGACGT    37800
          L  A  R  Q  T  P  R  P  A  A  A  A  P  S  V  G  K  R  D  V
                  .         .         .         .         .         .
37801    CGGCACGTGGTGCTACGCCCGCGGCTGGCGGCACGCCGCGCCCGCCGACCCCGCCCCGCT    37860
          G  T  W  C  Y  A  R  G  W  R  H  A  A  P  A  D  P  A  P  L
                  .         .         .         .         .         .
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37861  GACGGGCGGCTGCCTGCTGCTGGGCGACGGGGACACGGCGAAGGCCGTCGCGAGCCGGCT   37920
        T  G  G  C  L  L  L  G  D  G  D  T  A  K  A  V  A  S  R  L
                .         .         .         .         .         .
37921  GGAGGCCCTCGGCGTGCCCGTCACCACCGTCGGCGGCGGCCGACCGCCGGGCCCCGAGCG   37980
        E  A  L  G  V  P  V  T  T  V  G  G  G  R  P  P  G  P  E  R
                .         .         .         .         .         .
37981  GTACCGGGAACTCGTCGGCCCCGCCACCCGCCTGGCCGTCGACCTGTGGCCGCTGCGCGA   38040
        Y  R  E  L  V  G  P  A  T  R  L  A  V  D  L  W  P  L  R  D
                .         .         .         .         .         .
38041  CGCGTCCCACCGCGGCCGCGCCGCCGGCGCCGCCGGCGTACGGACCGCCCAGGACGCCGC   38100
        A  S  H  R  G  R  A  A  G  A  A  G  V  R  T  A  Q  D  A  A
                .         .         .         .         .         .
38101  GCTGCACAACCTGCTCCACCTCGCCCGGGCCTTCGGCGCGCTGGAGGAGCGCCACCCCGC   38160
        L  H  N  L  L  H  L  A  R  A  F  G  A  L  E  E  R  H  P  A
                .         .         .         .         .         .
38161  CCGCGTCGTGACCGTGACCACCGGTGCCCACGACGTGCTCGGCGACGACCTCGCCCACCC   38220
        R  V  V  T  V  T  T  G  A  H  D  V  L  G  D  D  L  A  H  P
                .         .         .         .         .         .
38221  CGAGCACGCCACCGTCCCGGCCGCGGCCAAGGTGATCCCCCGGGAGTACCCGTGGATCGC   38280
        E  H  A  T  V  P  A  A  A  K  V  I  P  R  E  Y  P  W  I  A
                .         .         .         .         .         .
38281  CTGCACCGCCCTGGACGTGGAGCCGGGCCTGGACGCCGAGCGGCTGGCGGACCTGATCGT   38340
        C  T  A  L  D  V  E  P  G  L  D  A  E  R  L  A  D  L  I  V
                .         .         .         .         .         .
38341  CCGGGAACTCGGCGCGGCGCGCGAGACCACCGTCACCGCCTGCCGCGGCCGACGCCGCTT   38400
        R  E  L  G  A  A  R  E  T  T  V  T  A  C  R  G  R  R  R  F
                .         .         .         .         .         .
38401  CACCCCCTGCCCCGTCCGGCAGCCCCTCCCCGCCGCACCGGAACGCCCGGCGGTCCGGCC   38460
        T  P  C  P  V  R  Q  P  L  P  A  A  P  E  R  P  A  V  R  P
                .         .         .         .         .         .
38461  CGGCGGCGTCTACCTCGTCTGCGGCGGCCTCGGCGGCATCGGCCTCCACCTCGCCGAGTA   38520
        G  G  V  Y  L  V  C  G  G  L  G  G  I  G  L  H  L  A  E  Y
                .         .         .         .         .         .
38521  CCTGGGCCGCGCCCGCACCACCGTCGTCCTCACCCACCGGCGGCCCTTTCCCGCCCCCGG   38580
        L  G  R  A  R  T  T  V  V  L  T  H  R  R  P  F  P  A  P  G
                .         .         .         .         .         .
38581  CGCGTGGGACGGGCTGCCCGCGGGACACCCGGAGGCGGCCGTCGTCCGGCGGCTGCGCTC   38640
        A  W  D  G  L  P  A  G  H  P  E  A  A  V  V  R  R  L  R  S
                .         .         .         .         .         .
38641  CCTCGCCGCCACCGGCGCCACGGTCGTCGTCCGCCGGGCCGACCTCACCGACCACGACGC   38700
        L  A  A  T  G  A  T  V  V  V  R  R  A  D  L  T  D  H  D  A
                .         .         .         .         .         .
38701  GATGCGCGCCCTCGCGGACGAGGTGGAACAGGCCCACGGCCCCGTCCGGGGGGTGGTGCA   38760
        M  R  A  L  A  D  E  V  E  Q  A  H  G  P  V  R  G  V  V  H
                .         .         .         .         .         .
38761  CGCGGCCGGGGTGCCCGACACCGCCGGCATGATCCAGCGTCGCGACCGAGCCGGCACGGA   38820
        A  A  G  V  P  D  T  A  G  M  I  Q  R  R  D  R  A  G  T  D
                .         .         .         .         .         .
38821  CGCCGCCCTCGCCGCCAAACTGACCGGCACCCTCGTCCTGGACGAGGTGTTCGCCCACCG   38880
        A  A  L  A  A  K  L  T  G  T  L  V  L  D  E  V  F  A  H  R
                .         .         .         .         .         .
38881  CGACCTCGACTTCCTCGTCCTGTGCTCCTCGATCGGCACCGTGCTGCACAAGCTGAAGTT   38940
        D  L  D  F  L  V  L  C  S  S  I  G  T  V  L  H  K  L  K  F
                .         .         .         .         .         .
38941  CGGCGAGGTCGGCTACGTGGCGGGCAACGAGTTCCTCGACGCCTATGCCGCCCACCGCGC   39000
        G  E  V  G  Y  V  A  G  N  E  F  L  D  A  Y  A  A  H  R  A
                .         .         .         .         .         .
39001  GGCCCGCCGCCCCGGCAGAACCCTGTCGATCGCCTGGACCGACTGGCGGGAGTCGGGCAT   39060
        A  R  R  P  G  R  T  L  S  I  A  W  T  D  W  R  E  S  G  M
                .         .         .         .         .         .
39061  GTGGGCCGCCGCCCAGCGCCGTCTGACCGAGCGCTACGGCACCGGCGCCGACCTGCCCGT   39120
        W  A  A  A  Q  R  R  L  T  E  R  Y  G  T  G  A  D  L  P  V
                .         .         .         .         .         .
39121  ACCGCCCGGGGGCGACCTGCTCGGCGCGATCAGCCCCGAGGAGGGCGTCGACGTCTTCGC   39180
        P  P  G  G  D  L  L  G  A  I  S  P  E  E  G  V  D  V  F  A
                .         .         .         .         .         .
39181  CCGGCTGCTCGCCGCCGACACCGGCCCGAACGTCATCGTGTCGGCCCAGGACCTCGACGA   39240
        R  L  L  A  A  D  T  G  P  N  V  I  V  S  A  Q  D  L  D  E
                .         .         .         .         .         .
39241  ACTCCTCGCGCGGCACGCGGCGTACACCACCGACGACCACCTCGCCGCCCTCGGCGACCT   39300
        L  L  A  R  H  A  A  Y  T  T  D  D  H  L  A  A  L  G  D  L
                .         .         .         .         .         .
39301  GAGGATCGCCGCCGCCCGGGACCGCTCCGCGCCCGCCGCGCCGTACGCGGCCCCCCACAC   39360
        R  I  A  A  A  R  D  R  S  A  P  A  A  P  Y  A  A  P  H  T
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                 .         .         .         .         .         .
39361   GCCCGCCCAGCGGCGGATCGCCGGCTGGTACCGCGACCTGCTCGGCGTCGAACACGTCGG   39420
         P  A  Q  R  R  I  A  G  W  Y  R  D  L  L  G  V  E  H  V  G
                 .         .         .         .         .         .
39421   CCTCGACGACGACTTCTTCGCGCTCGGCGGGGACTCGCTGCTCGCCCTGCGCCTGCTGTC   39480
         L  D  D  D  F  F  A  L  G  G  D  S  L  L  A  L  R  L  L  S
                 .         .         .         .         .         .
39481   GCAGCTGCGGGACGCCTACGGGGTGGAGATCTCCGTCGCCCGCATGTTCGACGAGCCCAC   39540
         Q  L  R  D  A  Y  G  V  E  I  S  V  A  R  M  F  D  E  P  T
                 .         .         .         .         .         .
39541   GGTGGCGGCGCTGGCCGCCGCCACCGGCCCGCCGCCGGAAGAGACGCCCGGCCAGGAAGA   39600
         V  A  A  L  A  A  A  T  G  P  P  P  E  E  T  P  G  Q  E  E
                 .         .         .         .         .         .
39601   GGTGGTGCTGTGACCACGCCCCGCATCACCGACCTGCTCACCGAGCTCCGCGGCCGGCAG   39660
         V  V  L  *
                 M  T  T  P  R  I  T  D  L  L  T  E  L  R  G  R  Q     (orf23)
                 .         .         .         .         .         .
39661   GTGACCCTCACGGCCGACGGGGACCGGCTGCACTGCCGCGCGCCCCGGGGCGCGCTCACC   39720
        V  T  L  T  A  D  G  D  R  L  H  C  R  A  P  R  G  A  L  T
                 .         .         .         .         .         .
39721   GACGAGCTCCTCGCCACCATCCGCGCCCGCCGCGACGAACTCCTCGCCCACCTGCGCGCC   39780
        D  E  L  L  A  T  I  R  A  R  R  D  E  L  L  A  H  L  R  A
                 .         .         .         .         .         .
39781   GACCGCCGCATCCCGCGCCACGACGGGCCCGCGCCGCTGTCCTTCGCCCAGGAACGGCTC   39840
        D  R  R  I  P  R  H  D  G  P  A  P  L  S  F  A  Q  E  R  L
                 .         .         .         .         .         .
39841   TGGCTCCTCCACCAGTTCCACCCGCACGACAGCGCCTACAACATCCCCCTGCACATCGCC   39900
        W  L  L  H  Q  F  H  P  H  D  S  A  Y  N  I  P  L  H  I  A
                 .         .         .         .         .         .
39901   CTGCGCGGGCCCCTGAACCCGGCCGCCCTGCGCGCCGCCCTGGCCGAGGTGGTACGGCGG   39960
        L  R  G  P  L  N  P  A  A  L  R  A  A  L  A  E  V  V  R  R
                 .         .         .         .         .         .
39961   CACGACGTCCTGCGCACCCGGTACGCCATCAGCCGCGGCCTGCCCCGGCCCGTCGTCGAA   40020
        H  D  V  L  R  T  R  Y  A  I  S  R  G  L  P  R  P  V  V  E
                 .         .         .         .         .         .
40021   CCGGCCCACACGCCGCCGCTGCCCCTGACCGACCTGACCGGGCTCCCCGCACACCACCGG   40080
        P  A  H  T  P  P  L  P  L  T  D  L  T  G  L  P  A  H  H  R
                 .         .         .         .         .         .
40081   GACGCCGAACTCGCCCGGCTGGCCGCCCAGGAGGCCAGGCGGCCCTTCGACCTCGCCCAG   40140
        D  A  E  L  A  R  L  A  A  Q  E  A  R  R  P  F  D  L  A  Q
                 .         .         .         .         .         .
40141   GGCCCGGTGCTGCGGGCCCGGCTCCTCCGAACGGCCCCCGAGGAGCACCGGCTGCTGCTG   40200
        G  P  V  L  R  A  R  L  L  R  T  A  P  E  E  H  R  L  L  L
                 .         .         .         .         .         .
40201   ACCCGCCATCACATCGCCAGCGACGGCTGGTCGCTCGACATCCTGCTCCGCGAACTGGGC   40260
        T  R  H  H  I  A  S  D  G  W  S  L  D  I  L  L  R  E  L  G
                 .         .         .         .         .         .
40261   ACGTTCTACCGGGCAGGGCGGGACGGCACACCCGCCGGCCTCGACGCCCTGCCGCTGCGG   40320
        T  F  Y  R  A  G  R  D  G  T  P  A  G  L  D  A  L  P  L  R
                 .         .         .         .         .         .
40321   TACGCCGACTTCGCCGCGTACCAGCGCGAACAGGCCGAACGGCCGGAGACGGCCGAGCGG   40380
        Y  A  D  F  A  A  Y  Q  R  E  Q  A  E  R  P  E  T  A  E  R
                 .         .         .         .         .         .
40381   TCGACCCGCTGGGCACGGCACCTGAGGGGCGCCCCCGCGACACTCGACGTCCTCGGGCCC   40440
        S  T  R  W  A  R  H  L  R  G  A  P  A  T  L  D  V  L  G  P
                 .         .         .         .         .         .
40441   CCGCCCGCCGAACCCTCCCACGCGCCGGCCGGCACCGTACGGACGGACCTTCCCGCCGCC   40500
        P  P  A  E  P  S  H  A  P  A  G  T  V  R  T  D  L  P  A  A
                 .         .         .         .         .         .
40501   CTCGTCACCGGCCTGCGGCAGCTGGGCGGCCGGGCCCGCACCACGCTCTTCCCGCTCCTG   40560
        L  V  T  G  L  R  Q  L  G  G  R  A  R  T  T  L  F  P  L  L
                 .         .         .         .         .         .
40561   CTGAGCGCCTTCGGCCTCGCCCTGGCCGGCCCGCCCGGCCCGTACGACGTCATGGTCGGC   40620
        L  S  A  F  G  L  A  L  A  G  P  P  G  P  Y  D  V  M  V  G
                 .         .         .         .         .         .
40621   ATCCCCGTCGCCGGCCGGCCGCGCACCGAACTGGAGCCGCTCATCGGCTGCTTCGCGACC   40680
        I  P  V  A  G  R  P  R  T  E  L  E  P  L  I  G  C  F  A  T
                 .         .         .         .         .         .
40681   ATCGCGCCGATGCGGCTGACGAGCGACGGGACCGAGCCGCTGACCCGGCTCGCCGCCCGC   40740
        I  A  P  M  R  L  T  S  D  G  T  E  P  L  T  R  L  A  A  R
                 .         .         .         .         .         .
40741   GCCCAGCAGCACGTCCAGGACGCGCTGGACGGACCCGACGTCCCCTTCGAGCGGCTCGTG   40800
        A  Q  Q  H  V  Q  D  A  L  D  G  P  D  V  P  F  E  R  L  V
                 .         .         .         .         .         .
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40801   CACGCGCTGCGTCCGGAGCGGGACCTCGCGGAGAACCCCCTGTTCTCGGCGTCGTTCGCC   40860
        H  A  L  R  P  E  R  D  L  A  E  N  P  L  F  S  A  S  F  A
                 .         .         .         .         .         .
40861   TTCCAGAACACCCCGCGGACCGCCGTGCGCCTCCCCGGCCTGGACGCCGAGGTGCTGCCC   40920
        F  Q  N  T  P  R  T  A  V  R  L  P  G  L  D  A  E  V  L  P
                 .         .         .         .         .         .
40921   TCGCCGCCCGTGGCCCCCAAGTTCCCGCTGGCCCTCACCGCGACGGCGCGGGCCGACGGC   40980
        S  P  P  V  A  P  K  F  P  L  A  L  T  A  T  A  R  A  D  G
                 .         .         .         .         .         .
40981   GGAATGGGCCTGGAGCTGGAGTTCGACCGGGACCGGATCGCCGAGCCGGTCGCGCGGGGG   41040
        G  M  G  L  E  L  E  F  D  R  D  R  I  A  E  P  V  A  R  G
                 .         .         .         .         .         .
41041   ATCCTCACGTCCTTCCACGCCGCCCTCGCCCGCGCGGTCGCCGACCCCGAGGCCCCGGCG   41100
        I  L  T  S  F  H  A  A  L  A  R  A  V  A  D  P  E  A  P  A
                 .         .         .         .         .         .
41101   GCGCCCGTACCGGCCGCCGCCGTGGACCGGCGGCCCGGGCGCGAAGGACACGAGTGCCTC   41160
        A  P  V  P  A  A  A  V  D  R  R  P  G  R  E  G  H  E  C  L
                 .         .         .         .         .         .
41161   CACGAGCCGGTGGCGCGGGCGGCGGCACGCCACCCCGACGCCGTCGCCGTCAGCTGCGGC   41220
        H  E  P  V  A  R  A  A  A  R  H  P  D  A  V  A  V  S  C  G
                 .         .         .         .         .         .
41221   GGCACCCAGCTCAGCTACGGGGCGCTCGACACCCGCGCCGAACGGCTGGCCGCGGTGCTG   41280
        G  T  Q  L  S  Y  G  A  L  D  T  R  A  E  R  L  A  A  V  L
                 .         .         .         .         .         .
41281   CGCGCCCACGGCGCCGGCCCCGAGCGGCTGGTGGCCCTGTGCCTGCCCACCGGCCCCGAA   41340
        R  A  H  G  A  G  P  E  R  L  V  A  L  C  L  P  T  G  P  E
                 .         .         .         .         .         .
41341   TGGGTCGTCGGCGCCCTCGCCATCCTCAAGTCCGGCGCCGCCTACCTGCCGCTCGACCCC   41400
        W  V  V  G  A  L  A  I  L  K  S  G  A  A  Y  L  P  L  D  P
                 .         .         .         .         .         .
41401   GGCGACCCGGCCGAGCGCCGCGCCTCCGTCGCCGCCGACGCGGGAGCGACGCTGATCGTC   41460
        G  D  P  A  E  R  R  A  S  V  A  A  D  A  G  A  T  L  I  V
                 .         .         .         .         .         .
41461   TCCGACACCGCGCTTCCCCCGCTCCACCGCGTCGACGTCACGGCCACCCTCCCGGACGGC   41520
        S  D  T  A  L  P  P  L  H  R  V  D  V  T  A  T  L  P  D  G
                 .         .         .         .         .         .
41521   GCCCCCGAGCCCACCGCCCGGGCCGTCCTGCCCGGCAACCTCGCCTACGCCGTCTACACC   41580
        A  P  E  P  T  A  R  A  V  L  P  G  N  L  A  Y  A  V  Y  T
                 .         .         .         .         .         .
41581   TCCGGCTCCACCGGCGGCCCCAAGGGCGTGCTCGTCACCCATGCCAACGTCACCGGGCTC   41640
        S  G  S  T  G  G  P  K  G  V  L  V  T  H  A  N  V  T  G  L
                 .         .         .         .         .         .
41641   CTGGCCGCGTGCCGTGAGGCCCTGCCCGCCCTGGACGCCCCCCGGACCTGGTCGGCGACC   41700
        L  A  A  C  R  E  A  L  P  A  L  D  A  P  R  T  W  S  A  T
                 .         .         .         .         .         .
41701   CACTCGCCGGCCTTCGACTTCTCCGTCTGGGAGGTCTGGGGCCCGCTGACCGCCGGCGGA   41760
        H  S  P  A  F  D  F  S  V  W  E  V  W  G  P  L  T  A  G  G
                 .         .         .         .         .         .
41761   CGCCTCGTCCTCGTGCCCCCGGACGTGGCCCGGGCCCCGGACGAACTGTGGGACACCCTC   41820
        R  L  V  L  V  P  P  D  V  A  R  A  P  D  E  L  W  D  T  L
                 .         .         .         .         .         .
41821   CGCGACGAACAGGTCGAAGTCCTCAGCCAGACCCCCAGCGCGTTCCACCACCTCCTGCCC   41880
        R  D  E  Q  V  E  V  L  S  Q  T  P  S  A  F  H  H  L  L  P
                 .         .         .         .         .         .
41881   ACCGCCGTGCGCCGGGCGGCCCAGGCCACCGCGCTCGAACTCGTCGTCCTGGGCGGCGAG   41940
        T  A  V  R  R  A  A  Q  A  T  A  L  E  L  V  V  L  G  G  E
                 .         .         .         .         .         .
41941   GCGTGCGAGCCCGCCCGTCTGACGCCTTGGTGGGACGCCCTGGGCGACCGGCGCCCGGCC   42000
        A  C  E  P  A  R  L  T  P  W  W  D  A  L  G  D  R  R  P  A
                 .         .         .         .         .         .
42001   GTGGTCAACATGTACGGCATCACCGAGAACACCATCCACGTCACCGTCCGCCGGATGACG   42060
        V  V  N  M  Y  G  I  T  E  N  T  I  H  V  T  V  R  R  M  T
                 .         .         .         .         .         .
42061   GCGGCGGACCGGTCGGGCAGTCCCGTCGGCCGGCCGCTGCCGGGGCAGCGCGCCGACCTT   42120
        A  A  D  R  S  G  S  P  V  G  R  P  L  P  G  Q  R  A  D  L
                 .         .         .         .         .         .
42121   CTCGACCCCCACGGCCGGCCCGTCGCGCCGGGCGGGCGGGGCGAACTGTTCGTCGGCGGC   42180
        L  D  P  H  G  R  P  V  A  P  G  G  R  G  E  L  F  V  G  G
                 .         .         .         .         .         .
42181   GTCGGACTGGCCCGCGGCTACCTCGGCCGGCCCGGCCTCACCGCCCGGAGCTTCCTGCCG   42240
        V  G  L  A  R  G  Y  L  G  R  P  G  L  T  A  R  S  F  L  P
                 .         .         .         .         .         .
42241   GACGACACCCCCGGCTGGCCGGGCGCGCGCCGCTACCGCTCCGGAGACCTGGCCCGGCTG   42300
        D  D  T  P  G  W  P  G  A  R  R  Y  R  S  G  D  L  A  R  L
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                 .         .         .         .         .         .
42301   CTGCCCGACGGCGGCCTGGACTACGCGGGCCGCTCCGACGCACAGGTCAAGGTCCGCGGC   42360
        L  P  D  G  G  L  D  Y  A  G  R  S  D  A  Q  V  K  V  R  G
                 .         .         .         .         .         .
42361   TACCGCGTCGAGCCCGCCGAGACCGAAGCCGCCGCGCTGACCCATCCCGCCGTGCGCCAC   42420
        Y  R  V  E  P  A  E  T  E  A  A  A  L  T  H  P  A  V  R  H
                 .         .         .         .         .         .
42421   TGCGTGGTCGTGCCACGCGGCGACGGCGACCGGCGCCATCTCGCGGCGTACGTCGTCGCC   42480
        C  V  V  V  P  R  G  D  G  D  R  R  H  L  A  A  Y  V  V  A
                 .         .         .         .         .         .
42481   GACACCCGCGCCTGCGACGGGCCCGGGCTCCGCACCCACCTGGCCGAGCGGCTGCCCCGC   42540
        D  T  R  A  C  D  G  P  G  L  R  T  H  L  A  E  R  L  P  R
                 .         .         .         .         .         .
42541   CACCTGGTGCCGGCCTCGGTGGTCTTCCTGAAGCGGATCCCGCTGACCCGCAACGGCAAG   42600
        H  L  V  P  A  S  V  V  F  L  K  R  I  P  L  T  R  N  G  K
                 .         .         .         .         .         .
42601   CTCGACGTGGCGGCCTTGCCCGACCCGGCCGCCCACCGCGCACCCGCCCGCGAACGCCCG   42660
        L  D  V  A  A  L  P  D  P  A  A  H  R  A  P  A  R  E  R  P
                 .         .         .         .         .         .
42661   CGCACCGCGACCGAACGGACCCTCACCCGGCTGCTCGCCGCCCTCCTGAAGGCGCCACCG   42720
        R  T  A  T  E  R  T  L  T  R  L  L  A  A  L  L  K  A  P  P
                 .         .         .         .         .         .
42721   GAGACCATCGGGACGCACGACAACCTCTTCGACCTGGGCGGCGACTCCCTGACGGTCACC   42780
        E  T  I  G  T  H  D  N  L  F  D  L  G  G  D  S  L  T  V  T
                 .         .         .         .         .         .
42781   CAGTTCCACTCCCGGGTGGTGGAGGAGTTCGCCGTGGACCTCCCGGTGCGCCGGGTCTAC   42840
        Q  F  H  S  R  V  V  E  E  F  A  V  D  L  P  V  R  R  V  Y
                 .         .         .         .         .         .
42841   CAGGCCCTCGACATCGCGACGCTCGCCGTGACCGTGGACGACTTCCGGCGCCGCGCCGAA   42900
        Q  A  L  D  I  A  T  L  A  V  T  V  D  D  F  R  R  R  A  E
                 .         .         .         .         .         .
42901   CGCACCGCGGTACTGCGCGCCCTCGCGGCGGCGGAGGCGATGGAACCCGGCGGTACGGCG   42960
        R  T  A  V  L  R  A  L  A  A  A  E  A  M  E  P  G  G  T  A
                 .         .         .         .         .         .
42961   GGGGAGTCCGGCGGTAATCCGGAGGAGTCCGCCGCTACGGCGCGGGGGCCCGCCGTCGCG   43020
        G  E  S  G  G  N  P  E  E  S  A  A  T  A  R  G  P  A  V  A
                 .         .         .         .         .         .
43021   GCGAACGAACCCGGCGCTGCGGCGCGTGAGTCCGGCGCCGCGCCGGTGGAGCCCGCCGTC   43080
        A  N  E  P  G  A  A  A  R  E  S  G  A  A  P  V  E  P  A  V
                 .         .         .         .         .         .
43081   GCAGTACAGGAGTCCGCCGCTACGAAGGGGGAGCCCGGCACCGCAGCGAATGAACTCGGC   43140
        A  V  Q  E  S  A  A  T  K  G  E  P  G  T  A  A  N  E  L  G
                 .         .         .         .         .         .
43141   GCTGAGGCACGGGAGCCCGGCACCGCAGCGCAGGAACCCGGCACCGACCCCCGGCCACCC   43200
        A  E  A  R  E  P  G  T  A  A  Q  E  P  G  T  D  P  R  P  P
                 .         .         .         .         .         .
43201   GCCGCCACACCGCAGGACCCCCGCACCACACCGCAGGAAGGACAGCCGTGCCCGCGTCCC   43260
        A  A  T  P  Q  D  P  R  T  T  P  Q  E  G  Q  P  C  P  R  P
                 .         .         .         .         .         .
43261   GAATGAGCCGGCCGGCCGGCATCGTCGACATCGCGCGCCGTCACGCCGAGCGCACCCCCG   43320
          M  S  R  P  A  G  I  V  D  I  A  R  R  H  A  E  R  T  P  A   (orf22)
        E  *
                 .         .         .         .         .         .
43321   CCCGTCCCGCGTACGCGTTCCTGCCCGACGGCGAGACGGAGAGCGTCCGCTTCTCCTTCG   43380
          R  P  A  Y  A  F  L  P  D  G  E  T  E  S  V  R  F  S  F  A
                 .         .         .         .         .         .
43381   CCGACATCGACCGGCGGGCCCGCGCCGTGGCCGCCGTCCTCCAGGACCGCGGCCTGGCCG   43440
          D  I  D  R  R  A  R  A  V  A  A  V  L  Q  D  R  G  L  A  G
                 .         .         .         .         .         .
43441   GGGAGCGGGTCCTGGTCGCCTATCCCTCCGGGCCCGAGTACGTCCAGGCGTTCCTGGGCT   43500
          E  R  V  L  V  A  Y  P  S  G  P  E  Y  V  Q  A  F  L  G  C
                 .         .         .         .         .         .
43501   GCCTGTACGCGGGCGTGGTCGCCGTCCCCTGCGACGAGCCGCGCTCCGGCCCGAGCGCGG   43560
          L  Y  A  G  V  V  A  V  P  C  D  E  P  R  S  G  P  S  A  E
                 .         .         .         .         .         .
43561   AACGGCTCGCCGGGATCCGCGCCGACGCCCGCCCCGCCCTGGCCCTGACCGCCGGCGCCC   43620
          R  L  A  G  I  R  A  D  A  R  P  A  L  A  L  T  A  G  A  P
                 .         .         .         .         .         .
43621   CCGAGGCCGGGCTCGCCGGCCTGGCCACCCTGGACGTGGCCGGCGTCCCCGACTCCGCCG   43680
          E  A  G  L  A  G  L  A  T  L  D  V  A  G  V  P  D  S  A  A
                 .         .         .         .         .         .
43681   CCGGGGCCTGGACCGACCCCGTCGCGGGACCGGACGCCCTGGCCTTCCTCCAGTACACCT   43740
          G  A  W  T  D  P  V  A  G  P  D  A  L  A  F  L  Q  Y  T  S
                 .         .         .         .         .         .
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43741  CCGGATCGACCCGCCGCCCCCGCGGCGTCATGGTCGGCCACGGCAATCTGCTGGCCAACG   43800
         G  S  T  R  R  P  R  G  V  M  V  G  H  G  N  L  L  A  N  E
                .         .         .         .         .         .
43801  AGCGCTGCATCGCCGCCGCCTGCGGCCACGACCGGGACTCCACCTTCGTGGGATGGGCGC   43860
         R  C  I  A  A  A  C  G  H  D  R  D  S  T  F  V  G  W  A  P
                .         .         .         .         .         .
43861  CGTTCTTCCACGACATGGGCCTGGTCGCCAACCTCCTCCAGCCCCTCTACCTCGGGTCCC   43920
         F  F  H  D  M  G  L  V  A  N  L  L  Q  P  L  Y  L  G  S  L
                .         .         .         .         .         .
43921  TGTCGGTGCTGATGCCGCCGATGGCCTTCCTCCAGCGCCCGGCCCGCTGGCTGCGGGCCG   43980
         S  V  L  M  P  P  M  A  F  L  Q  R  P  A  R  W  L  R  A  V
                .         .         .         .         .         .
43981  TCTCCCGCTACCGGGCGCACACCAGCGGCGGCCCCAACTTCGCCTACGACCTGTGTGTCG   44040
         S  R  Y  R  A  H  T  S  G  G  P  N  F  A  Y  D  L  C  V  D
                .         .         .         .         .         .
44041  ACCGGGTCGGCGAGGACGAGCGGGCCGGACTGGACCTGTCGGGCTGGAAGGTCGCCTACA   44100
         R  V  G  E  D  E  R  A  G  L  D  L  S  G  W  K  V  A  Y  N
                .         .         .         .         .         .
44101  ACGGCGCGGAACCTGTACGGGCCGACACCCTGCGACGGTTCACCGACCGCTTCGCCCCCC   44160
         G  A  E  P  V  R  A  D  T  L  R  R  F  T  D  R  F  A  P  H
                .         .         .         .         .         .
44161  ACGGCTTCACCCCCGGCGCGCACTTCCCGACCTACGGGCTCGCCGAGGCGACCCTGCTCG   44220
         G  F  T  P  G  A  H  F  P  T  Y  G  L  A  E  A  T  L  L  V
                .         .         .         .         .         .
44221  TCGCCACCGGCCCCAAGGGAGTGCCGCCCCGCACCCTGACCGCCGACCGCGCCGCCCTGC   44280
         A  T  G  P  K  G  V  P  P  R  T  L  T  A  D  R  A  A  L  R
                .         .         .         .         .         .
44281  GCGCCGGCCGGCTCCGGCCCGCCGGGCCCGGCGAGGCCGGCCTGGAACTGGTCGGCAACG   44340
         A  G  R  L  R  P  A  G  P  G  E  A  G  L  E  L  V  G  N  G
                .         .         .         .         .         .
44341  GCACCGCCGGCCTCGACACCACCCTCCGGATCGTCGACCCCGCGACCGCGCGGGAGTGCC   44400
         T  A  G  L  D  T  T  L  R  I  V  D  P  A  T  A  R  E  C  P
                .         .         .         .         .         .
44401  CGCCCGGAGAGGTCGGCGAGGTCTGGGTGCGCGGCCCGGGCGTGGCACGCGGCTACTTCG   44460
         P  G  E  V  G  E  V  W  V  R  G  P  G  V  A  R  G  Y  F  G
                .         .         .         .         .         .
44461  GCCGCCCGCGCGAGTCCGCGCCGCTGCTCGCCGCCCGCCTGCCCGGCGGCGAAGGACCGT   44520
         R  P  R  E  S  A  P  L  L  A  A  R  L  P  G  G  E  G  P  Y
                .         .         .         .         .         .
44521  ACCTGCGGACCGGGGACCTGGGCGCCCTGCACGACGGGGAACTCTTCCTCACCGGACGCC   44580
         L  R  T  G  D  L  G  A  L  H  D  G  E  L  F  L  T  G  R  H
                .         .         .         .         .         .
44581  ACAAGGACCTCATCGTCATCCGCGGCCAGAACCACCACCCGCACGACCTCGAACGGACCG   44640
         K  D  L  I  V  I  R  G  Q  N  H  H  P  H  D  L  E  R  T  A
                .         .         .         .         .         .
44641  CCGAGCAGGCCCACCCGGCGCTCCGCCCGACCTGCGCCGCCGCGTTCGCGGTGCCCGGGG   44700
         E  Q  A  H  P  A  L  R  P  T  C  A  A  A  F  A  V  P  G  D
                .         .         .         .         .         .
44701  ACGGCGCGGAGCGGCTCGTGCTCGTCTGCGAACTCACCTCCTACCGCGCCGTCGACCCGG   44760
         G  A  E  R  L  V  L  V  C  E  L  T  S  Y  R  A  V  D  P  A
                .         .         .         .         .         .
44761  CCGCCGTCGCCGAGGCCGTCCGGGCCGCGCTCGCCGCGCGGCACGGCGTCGCCCCGCACA   44820
         A  V  A  E  A  V  R  A  A  L  A  A  R  H  G  V  A  P  H  T
                .         .         .         .         .         .
44821  CGCTGGTGGTGCTGCGCCGCGGCGGCATCCCCAAGACCACCAGCGGAAAGGTGCGGCGCG   44880
         L  V  V  L  R  R  G  G  I  P  K  T  T  S  G  K  V  R  R  G
                .         .         .         .         .         .
44881  GCCACTGCCGGACGGCCTACCTCGACGGAACGCTCCCCGTTCACACGGCCGTCCGCCTCC   44940
         H  C  R  T  A  Y  L  D  G  T  L  P  V  H  T  A  V  R  L  P
                .         .         .         .         .         .
44941  CGGCGGGGGAGGAGGGCACCGAGGCCCTTCCCCTGACCACGGACCCCGGTCGGCTGGCCA   45000
         A  G  E  E  G  T  E  A  L  P  L  T  T  D  P  G  R  L  A  T
                .         .         .         .         .         .
45001  CGGCGCTGCGCGACCTGGCCGCCGCCCACGCGGGCCTGGCCGGGCCCCTCCCCGGCACCG   45060
         A  L  R  D  L  A  A  A  H  A  G  L  A  G  P  L  P  G  T  D
                .         .         .         .         .         .
45061  ACGAGCCGGTGAGCGCCCTCGGCCTGGACTCGCTCGCCTCCCTGCGGCTCCACCACCACG   45120
         E  P  V  S  A  L  G  L  D  S  L  A  S  L  R  L  H  H  H  V
                .         .         .         .         .         .
45121  TCCAGTCCGCCTACGGCGTGACCCTGCCCGTCACCGCCCTGCTCGGCGACACCACTTACC   45180
         Q  S  A  Y  G  V  T  L  P  V  T  A  L  L  G  D  T  T  Y  R
                .         .         .         .         .         .
45181  GCCGGCTCGCGGAGCTGACGCTCGCCGCCCCCCGCCCGGCCCGGGCGCCCGAGGGGCAAG   45240
         R  L  A  E  L  T  L  A  A  P  R  P  A  R  A  P  E  G  Q  V
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                .         .         .         .         .         .
45241  TCACCGGCGTCTGGCGGCCGTTGACGCACGGGCAGCGCGCCCTGTGGTACGAACAGGCGC   45300
         T  G  V  W  R  P  L  T  H  G  Q  R  A  L  W  Y  E  Q  A  L
                .         .         .         .         .         .
45301  TCGCCCCGCACGCGGCCGCCTACCACCTCGTCCGCGCGCTGGCCCTCCGCGGCCCCGTCG   45360
         A  P  H  A  A  A  Y  H  L  V  R  A  L  A  L  R  G  P  V  D
                .         .         .         .         .         .
45361  ACGAGGAGGCCCTCGCCGAGGCGGTCCGCCGCGTCGTCCGCCGCCACCCCGCCCTGCGGA   45420
         E  E  A  L  A  E  A  V  R  R  V  V  R  R  H  P  A  L  R  T
                .         .         .         .         .         .
45421  CCCGCTTCGCGCTCCGCGACGGCGAACCGGCGCGCCGGACCGAGCCGTACGGACCGGAGC   45480
         R  F  A  L  R  D  G  E  P  A  R  R  T  E  P  Y  G  P  E  L
                .         .         .         .         .         .
45481  TGGACGTACGCGACGCCACCGGCCTGCCGGCGGACCGGCTCCGCGAACACCTGGCCGCGG   45540
         D  V  R  D  A  T  G  L  P  A  D  R  L  R  E  H  L  A  A  A
                .         .         .         .         .         .
45541  CGGGCGACCGCCCCTTCGACCTGGCCGCCGGCGACAGGCCCGTGAGGCTGACGCTCTACC   45600
         G  D  R  P  F  D  L  A  A  G  D  R  P  V  R  L  T  L  Y  R
                .         .         .         .         .         .
45601  GCACGGACGGCGGCCACATCCTGCTGCTGGTCGCCCACCACCTGGTCGCCGACTTCTGGT   45660
         T  D  G  G  H  I  L  L  L  V  A  H  H  L  V  A  D  F  W  S
                .         .         .         .         .         .
45661  CCCTCGTCGTCCTCCTGGGCGACCTCGCCCGGGCCCACGCGGGCGAGGACCTGCCGCCCG   45720
         L  V  V  L  L  G  D  L  A  R  A  H  A  G  E  D  L  P  P  A
                .         .         .         .         .         .
45721  CGCCGGAGGGGGACCCCGGCGACGAGGCGACGGACGCGGACCGGACGTACTGGCGGCACC   45780
         P  E  G  D  P  G  D  E  A  T  D  A  D  R  T  Y  W  R  H  R
                .         .         .         .         .         .
45781  GGCTCGCCGACGCGCCACCCGCCCTCGACCTGCCCACCGACCTCCCCCACCCCGCCGAGC   45840
         L  A  D  A  P  P  A  L  D  L  P  T  D  L  P  H  P  A  E  R
                .         .         .         .         .         .
45841  GCGGCTTCGCCGGCGCCACCCACGCCTTCCGGCTGCCCCCGGACCTCACCGCCCGGCTGA   45900
         G  F  A  G  A  T  H  A  F  R  L  P  P  D  L  T  A  R  L  T
                .         .         .         .         .         .
45901  CCGCCCTCTCCCGGGAACGGCACTGCACCCTCTTCACCACCCTCCTCGCCGCCCACCAGC   45960
         A  L  S  R  E  R  H  C  T  L  F  T  T  L  L  A  A  H  Q  L
                .         .         .         .         .         .
45961  TACTGCTCCACCGCCTGACCGGGCAGGACGACCTCGTCGTGGGCACCCTCCTCGCCCGCC   46020
         L  L  H  R  L  T  G  Q  D  D  L  V  V  G  T  L  L  A  R  R
                .         .         .         .         .         .
46021  GCGACACCGCCGAAGCGGCCGGCGCCGTCGGCTACCTGGTCAACCCGCTGCCGCTGCGCT   46080
         D  T  A  E  A  A  G  A  V  G  Y  L  V  N  P  L  P  L  R  S
                .         .         .         .         .         .
46081  CCGTACGGGAGCCGGGGGAGACCTTCACGGAACTGCTGCGCCGCACCCGGCGGACCGTGC   46140
         V  R  E  P  G  E  T  F  T  E  L  L  R  R  T  R  R  T  V  L
                .         .         .         .         .         .
46141  TGGACGCGGTCGCGCACGGCCGCCACCCCTTCGGGCCGCTCGTCTCCCGTCTCGCCCCCG   46200
         D  A  V  A  H  G  R  H  P  F  G  P  L  V  S  R  L  A  P  A
                .         .         .         .         .         .
46201  CGCGCACGCCCGGCCGCGCGCCGCTCCTGCAGAGCCTGTTCGTGCTCCAGCGCGAGTACG   46260
         R  T  P  G  R  A  P  L  L  Q  S  L  F  V  L  Q  R  E  Y  G
                .         .         .         .         .         .
46261  GCGACGAGGCGGACGGGTACCGCGCGCTCGCCCTGGGCGTCGGCGGCCGGCTGCGCGTCG   46320
         D  E  A  D  G  Y  R  A  L  A  L  G  V  G  G  R  L  R  V  G
                .         .         .         .         .         .
46321  GCGGACTCGACCTGGAGGCACTCGCGTTGCCGCGCCGCTGGTCGCAGCTCGACCTCTCGC   46380
         G  L  D  L  E  A  L  A  L  P  R  R  W  S  Q  L  D  L  S  L
                .         .         .         .         .         .
46381  TGAGCATGGCGCGGCTCGGGGACGGGCTGACGGGGGTGTGGGAGTACCGCACCGACCTGT   46440
         S  M  A  R  L  G  D  G  L  T  G  V  W  E  Y  R  T  D  L  F
                .         .         .         .         .         .
46441  TCACCGAGGCCACGGTCGCGGAGCTGAGCGAGGCGTTCGTCCACCTGCTGCGGGCGGCCG   46500
         T  E  A  T  V  A  E  L  S  E  A  F  V  H  L  L  R  A  A  V
                .         .         .         .         .         .
46501  TCGAGGACCCGGGCGCGCCCGTGGAGACGCTGCCGCTCACCGGCGGCCGGGAGACCGGGC   46560
         E  D  P  G  A  P  V  E  T  L  P  L  T  G  G  R  E  T  G  P
                .         .         .         .         .         .
46561  CGCGCCGCGGCCCGTCGGCGGCCCGGCCCGCCCTCCCGCTGCACCGGCTCGTGGCCGCGG   46620
         R  R  G  P  S  A  A  R  P  A  L  P  L  H  R  L  V  A  A  A
                .         .         .         .         .         .
46621  CGGCGCGCCGCGATCCCGCACGGACGGCGGTCGTCGCACTCGCCCCGGACGGCACCGCCC   46680
         A  R  R  D  P  A  R  T  A  V  V  A  L  A  P  D  G  T  A  H
                .         .         .         .         .         .
46681  ACCACATCAGCCACGGAGCCCTGCACCGCGCGGCCACCACCCTCGCCGCCCGGCTCCGCC   46740
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          H  I  S  H  G  A  L  H  R  A  A  T  T  L  A  A  R  L  R  R
                 .         .         .         .         .         .
46741   GGGAGGGCGCCGGCCCGGAGCGGCCCGTCGCCGTGCTCGTCGAGCGGGGCCCCTGGCTGC   46800
          E  G  A  G  P  E  R  P  V  A  V  L  V  E  R  G  P  W  L  P
                 .         .         .         .         .         .
46801   CCGTCGCCTACCTCGGCATCCTGCACGCCGGGGCCACCGTGCTGCCCCTGGACCCGGAGG   46860
          V  A  Y  L  G  I  L  H  A  G  A  T  V  L  P  L  D  P  E  D
                 .         .         .         .         .         .
46861   ACCCCCCGCACAGGCTCGCCCGGACGATCGCGAACTCGGGGGCGCGGCTGCTGCTCACCG   46920
          P  P  H  R  L  A  R  T  I  A  N  S  G  A  R  L  L  L  T  E
                 .         .         .         .         .         .
46921   AGACCGGGACCGCCTCGCGCGCGGCCGAGGCGGCCGGTCCCGGCGTACGCGCGCTGACCG   46980
          T  G  T  A  S  R  A  A  E  A  A  G  P  G  V  R  A  L  T  V
                 .         .         .         .         .         .
46981   TGCGTGAGGGTGCCACCGGCGGCGAGCGGTTCTCGGCGGACGTCCACCCCGAGCAGTCCG   47040
          R  E  G  A  T  G  G  E  R  F  S  A  D  V  H  P  E  Q  S  A
                 .         .         .         .         .         .
47041   CGTACCTGCTGTACACCTCCGGGTCGACGGGCGACCCCAAGGGCGTGCTCGTCCCGCACC   47100
          Y  L  L  Y  T  S  G  S  T  G  D  P  K  G  V  L  V  P  H  R
                 .         .         .         .         .         .
47101   GGGCCATCGTCAACCGCCTCCTGTGGATGCAGGAGACCTACCGGCTGCGCCCGGGGGAGC   47160
          A  I  V  N  R  L  L  W  M  Q  E  T  Y  R  L  R  P  G  E  R
                 .         .         .         .         .         .
47161   GGGTCCTGCACAAGACGCCGGTGACGTTCGACGTCTCGATGTGGGAGCTGCTGTGGCCGC   47220
          V  L  H  K  T  P  V  T  F  D  V  S  M  W  E  L  L  W  P  L
                 .         .         .         .         .         .
47221   TGACCGCCGGGGCGACCGTCGTCATGGCCCGGCCCGGGACCCACCGCGACCCCGCGCGAC   47280
          T  A  G  A  T  V  V  M  A  R  P  G  T  H  R  D  P  A  R  L
                 .         .         .         .         .         .
47281   TCGTCCGGCGGATCGCCCGCGAGGCCGTCACCACCGTGCACTTCGTCCCCTCGATGCTCA   47340
          V  R  R  I  A  R  E  A  V  T  T  V  H  F  V  P  S  M  L  T
                 .         .         .         .         .         .
47341   CCCCGTTCCTCACCGAGCTCGCCCGCGGCACGACGCGGCTGCCCGCGCTGCGGCGCGTGG   47400
          P  F  L  T  E  L  A  R  G  T  T  R  L  P  A  L  R  R  V  V
                 .         .         .         .         .         .
47401   TGTGCAGCGGGGAAGAGCTGCCCGCGGCCGCGGTGAACCGCGCCGCCGGACTCCTCGACG   47460
          C  S  G  E  E  L  P  A  A  A  V  N  R  A  A  G  L  L  D  A
                 .         .         .         .         .         .
47461   CCCGGCTGTACAACCTCTACGGCCCGACCGAAGCCGCCGTCGACGTCACCGCCTGGCCCT   47520
          R  L  Y  N  L  Y  G  P  T  E  A  A  V  D  V  T  A  W  P  C
                 .         .         .         .         .         .
47521   GCCGCCCGCCCGAGCCGGGGCCGGTGCCGATCGGCCTGCCCATCGCCAACACCACCACCG   47580
          R  P  P  E  P  G  P  V  P  I  G  L  P  I  A  N  T  T  T  E
                 .         .         .         .         .         .
47581   AGGTCCTCGACGGCCGGCTGCGCCCGCTGCCCCGCCCGGTGCCCGGCGAGCTGTACCTGG   47640
          V  L  D  G  R  L  R  P  L  P  R  P  V  P  G  E  L  Y  L  G
                 .         .         .         .         .         .
47641   GCGGCGCCTGCCTGGCCCATGGCTACCACCACGACCCGGCCCTGACCGCCGCGCGCTTCC   47700
          G  A  C  L  A  H  G  Y  H  H  D  P  A  L  T  A  A  R  F  L
                 .         .         .         .         .         .
47701   TTCCGGCCCCCGGCGGCGGGCGCCGCTACCGCACCGGGGACCTCGTCCGCCAACGGGCCG   47760
          P  A  P  G  G  G  R  R  Y  R  T  G  D  L  V  R  Q  R  A  D
                 .         .         .         .         .         .
47761   ACGGGGCACTGGTGTTCCGGGGACGCACGGACGACCAGGTGAAGATCGGCGGCATCCGGG   47820
          G  A  L  V  F  R  G  R  T  D  D  Q  V  K  I  G  G  I  R  V
                 .         .         .         .         .         .
47821   TCGAGCCCGGCGAGGTGGCGGAGGCGCTTCGGGCCCTGCCCGGCGTCGCCGACGCCGCGG   47880
          E  P  G  E  V  A  E  A  L  R  A  L  P  G  V  A  D  A  A  V
                 .         .         .         .         .         .
47881   TCGTCCCGCACGACGGGCGGCTGGCGGCGTACGCGGTCGCCGACCCGGTCGGCCCGGCCC   47940
          V  P  H  D  G  R  L  A  A  Y  A  V  A  D  P  V  G  P  A  P
                 .         .         .         .         .         .
47941   CGGCGGCGGACGCCCTGCGGGACGCGCTGCGCAGGCGGCTGCCCGGCCACCTGGTGCCCG   48000
          A  A  D  A  L  R  D  A  L  R  R  R  L  P  G  H  L  V  P  A
                 .         .         .         .         .         .
48001   CCGCCCTCACCCTGCTGGACCGGCTGCCCCTCACCCCGGCGGGCAAGCTCGACCGCCGGG   48060
          A  L  T  L  L  D  R  L  P  L  T  P  A  G  K  L  D  R  R  A
                 .         .         .         .         .         .
48061   CGCTGCCCCACCCGTCGGCCCCGCCCCCGGACGGCGGACGGCCGCCCACGACCGGGACCG   48120
          L  P  H  P  S  A  P  P  P  D  G  G  R  P  P  T  T  G  T  E
                 .         .         .         .         .         .
48121   AACGGCTCGTCGCCCGGGTGTGGGCCGAACGCCTCGGACGGGAAGTCGTCGGCGTGGACC   48180
          R  L  V  A  R  V  W  A  E  R  L  G  R  E  V  V  G  V  D  R
                 .         .         .         .         .         .
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48181   GGGACTTCTTCTCCCTGGGCGGCGACTCCGTCCGGGCCCTCGGCGTGACGGCGGCCCTGC   48240
         D  F  F  S  L  G  G  D  S  V  R  A  L  G  V  T  A  A  L  R
                 .         .         .         .         .         .
48241   GCGCCGCCGGGCTCCCGGTGACGGTCACCGACCTCCTGCGCCTGCCCACCGTGGCCGCCC   48300
         A  A  G  L  P  V  T  V  T  D  L  L  R  L  P  T  V  A  A  L
                 .         .         .         .         .         .
48301   TCGCCCGCCACGCCGACGAGCGGGCGGATCGCCGACCGGCGCGACAGGAGACGCCCCCCG   48360
         A  R  H  A  D  E  R  A  D  R  R  P  A  R  Q  E  T  P  P  G
                 .         .         .         .         .         .
48361   GGCCGTTCGCCCTCTGCCCGGAAGCCGCCGGCGTGCCCGGCCTGGAGGACGCCTACCCGA   48420
         P  F  A  L  C  P  E  A  A  G  V  P  G  L  E  D  A  Y  P  M
                 .         .         .         .         .         .
48421   TGTCGATGGCCCAGCGGGCCGTGCTCTTCCACCGTGACCACAACCCCGGCTACGAGGTCT   48480
         S  M  A  Q  R  A  V  L  F  H  R  D  H  N  P  G  Y  E  V  Y
                 .         .         .         .         .         .
48481   ACGTCACCAGCGTCGCCGTCTCCACGCCCCTGGACCGCACACGGCTCGCCGCGGCCGTGG   48540
         V  T  S  V  A  V  S  T  P  L  D  R  T  R  L  A  A  A  V  D
                 .         .         .         .         .         .
48541   ACCGGCTGCTGGACCGGCACGCCTATCTGCGGTCCTCCTTCGACCTCGTGTCCCACCCGG   48600
         R  L  L  D  R  H  A  Y  L  R  S  S  F  D  L  V  S  H  P  E
                 .         .         .         .         .         .
48601   AGCCCACCCAGCTCGTCTGGACCCACCTGCCCACCCCGCTCGAGGTGGTGGAGTCGTCCG   48660
         P  T  Q  L  V  W  T  H  L  P  T  P  L  E  V  V  E  S  S  D
                 .         .         .         .         .         .
48661   ACCCCGCCGGTTTCGACGCGTGGCTGCACGCCGAACGCAAGCGCCCCCTCGACGTCGGCA   48720
         P  A  G  F  D  A  W  L  H  A  E  R  K  R  P  L  D  V  G  T
                 .         .         .         .         .         .
48721   CCGGACCGCTGGCCCGGTTCACCGCGCACGACGCGGGAGCCGCCGGATTCCGGCTGACCG   48780
         G  P  L  A  R  F  T  A  H  D  A  G  A  A  G  F  R  L  T  V
                 .         .         .         .         .         .
48781   TCAGCAGCTTCGCCCTCGACGGCTGGTGCGTGGCCACCGTGCTCACCGAACTGCTCCGCG   48840
         S  S  F  A  L  D  G  W  C  V  A  T  V  L  T  E  L  L  R  D
                 .         .         .         .         .         .
48841   ACTACTGGTCCGCGCTGCGCGGCGCGCCCCTCAGCCTCCCGGCACCCGCCGCCTCCTACC   48900
         Y  W  S  A  L  R  G  A  P  L  S  L  P  A  P  A  A  S  Y  R
                 .         .         .         .         .         .
48901   GCGAGTTCGTCGCCCTCGAACGCGCCGCCCAACACGATCCGGCGCACCGGGAGTTCTGGC   48960
         E  F  V  A  L  E  R  A  A  Q  H  D  P  A  H  R  E  F  W  R
                 .         .         .         .         .         .
48961   GGACGGAGCTCGCCGGTGCCCGGCCGCATCCGCTGCCCCGCCGCCCGGTGCCACCGCCCG   49020
         T  E  L  A  G  A  R  P  H  P  L  P  R  R  P  V  P  P  P  G
                 .         .         .         .         .         .
49021   GGCCGGACGGGATCCGCCAGCACCGTCACGTCGTCCCCGTCGAGGACACCGTCGCCAAGG   49080
         P  D  G  I  R  Q  H  R  H  V  V  P  V  E  D  T  V  A  K  G
                 .         .         .         .         .         .
49081   GCCTGTCGGCGCTCGCCGGCGAGCTGGGTGTCGGGCTCAAACACGTTCTGCTCGGCGTCC   49140
         L  S  A  L  A  G  E  L  G  V  G  L  K  H  V  L  L  G  V  H
                 .         .         .         .         .         .
49141   ACCTGCGGGTCGTCCGGGCCCTGTCCGGCGACCCCGACGTCATCACGGCCGTGGAGACCC   49200
         L  R  V  V  R  A  L  S  G  D  P  D  V  I  T  A  V  E  T  H
                 .         .         .         .         .         .
49201   ACGGCCGCCTCGAACGGCACGACGGCGACCGCGTCCTCGGGGTGTTCAACAACATCCTGC   49260
         G  R  L  E  R  H  D  G  D  R  V  L  G  V  F  N  N  I  L  P
                 .         .         .         .         .         .
49261   CGCTGCGGCAGCGGGTGGACGGCGGGAGCTGGGCCGACCTGGCCCGCGCCGCGCACGCCG   49320
         L  R  Q  R  V  D  G  G  S  W  A  D  L  A  R  A  A  H  A  A
                 .         .         .         .         .         .
49321   CGGAGGCGCGGACGGGGGAGTACCGCCGCTATCCGCTGGCCCAGGCACAGCGCGACCACG   49380
         E  A  R  T  G  E  Y  R  R  Y  P  L  A  Q  A  Q  R  D  H  G
                 .         .         .         .         .         .
49381   GCGCGGCCGGGCTCTTCGACACCCTCTTCGTGTTCACCCACTTCCACCTCTACCGCGCGC   49440
         A  A  G  L  F  D  T  L  F  V  F  T  H  F  H  L  Y  R  A  L
                 .         .         .         .         .         .
49441   TGGCCGACCTGGACGGCATGGCGGTCTCCGACCTGCGGGCCCCCGACCAGACCTACGTAC   49500
         A  D  L  D  G  M  A  V  S  D  L  R  A  P  D  Q  T  Y  V  P
                 .         .         .         .         .         .
49501   CGCTCACCGCCCACTTCAACGTCGACGCCACGGACGGCGGCGGCCTGCGGCTGCTGCTGG   49560
         L  T  A  H  F  N  V  D  A  T  D  G  G  G  L  R  L  L  L  E
                 .         .         .         .         .         .
49561   AGTCGGACCCGCGGGAGTTCCCCGACGAGCAGGTCGCGGAGTTCGCCGCGTACTACCGCC   49620
         S  D  P  R  E  F  P  D  E  Q  V  A  E  F  A  A  Y  Y  R  R
                 .         .         .         .         .         .
49621   GCGCGCTGCGGGCCGCCGCCGACGCCCCGCACCGGCCGTACCGGGACACGCCGTTGACGG   49680
         A  L  R  A  A  A  D  A  P  H  R  P  Y  R  D  T  P  L  T  D
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                .         .         .         .         .         .
49681  ACCGGCCGGCCGGTCCGGCGCCGCACCGCGCGGAGCGCTCCGTCCACGCCCTGTTCGCGG   49740
        R  P  A  G  P  A  P  H  R  A  E  R  S  V  H  A  L  F  A  A
                .         .         .         .         .         .
49741  CCCCGGCCCGGAACCACCCGGACCGGATCGCGCTCGACGGCGAGGACGGGCCGGTCAGCC   49800
        P  A  R  N  H  P  D  R  I  A  L  D  G  E  D  G  P  V  S  H
                .         .         .         .         .         .
49801  ACGGCGCCCTGGCCCGGCGCGCCGCCCGCCTCGCCGGAACGCTGCGGGCCGCGGGCGCCG   49860
        G  A  L  A  R  R  A  A  R  L  A  G  T  L  R  A  A  G  A  G
                .         .         .         .         .         .
49861  GGCCGGACACCGTCGTCGGGATCTGGGCGCCGCGCCGCGCCGACGCCGTCGTGGCGCTGC   49920
        P  D  T  V  V  G  I  W  A  P  R  R  A  D  A  V  V  A  L  L
                .         .         .         .         .         .
49921  TGGCCGCCCTCCACGCCGGAGCCGCCTACCTGCCCCTGGACCCGGTCCACCCGCCCCGGC   49980
        A  A  L  H  A  G  A  A  Y  L  P  L  D  P  V  H  P  P  R  R
                .         .         .         .         .         .
49981  GGCAGCGGCAGGTGCTCACCGAGGCCGGCGCCCGCCTGCTCGTCCTGCCCGCCGGCCTCG   50040
        Q  R  Q  V  L  T  E  A  G  A  R  L  L  V  L  P  A  G  L  D
                .         .         .         .         .         .
50041  ACACCCCGCTCCGGGCCTGCGGCCTGCCCGTCGTGGCCCCGGACGACCTCGGCGCGCCCA   50100
        T  P  L  R  A  C  G  L  P  V  V  A  P  D  D  L  G  A  P  I
                .         .         .         .         .         .
50101  TCGCCCCCGTGTCCGTCCACCCGGAGCAGCTGGCGGCGGTCATGGCCACGTCCGGCTCCA   50160
        A  P  V  S  V  H  P  E  Q  L  A  A  V  M  A  T  S  G  S  T
                .         .         .         .         .         .
50161  CCGGGACGCCCAAGACGATCGGCGTCCCGCAGCGCGCCCTGGCCGGCTACCTCCGCTGGG   50220
        G  T  P  K  T  I  G  V  P  Q  R  A  L  A  G  Y  L  R  W  A
                .         .         .         .         .         .
50221  CGATCGGCCACTACCGCCTCGACGAGGAGACCGTCTCCCCGGTGCACTCCTCGCTGGGCT   50280
        I  G  H  Y  R  L  D  E  E  T  V  S  P  V  H  S  S  L  G  F
                .         .         .         .         .         .
50281  TCGACCTGACCGTCACCGCGCTGCTCGCACCGCTGGCCGCCGGCGGGCAGGCGCGGCTGA   50340
        D  L  T  V  T  A  L  L  A  P  L  A  A  G  G  Q  A  R  L  T
                .         .         .         .         .         .
50341  CCGACTCCGGCGACCCGGGTGCCCTCGGCGCGGCACTGGCCGCCGGCCACCACACCCTGC   50400
        D  S  G  D  P  G  A  L  G  A  A  L  A  A  G  H  H  T  L  L
                .         .         .         .         .         .
50401  TCAAGATCACCCCGGCCCATCTGGCCGCCCTCGCCCACCAGTTGGGCGCGCCGACCGCAC   50460
        K  I  T  P  A  H  L  A  A  L  A  H  Q  L  G  A  P  T  A  L
                .         .         .         .         .         .
50461  TGCGCACCGTCGTGGCCGGGGGCGAACCCCTGCACGCCGGCCACGTCCGCGCCCTCCGCG   50520
        R  T  V  V  A  G  G  E  P  L  H  A  G  H  V  R  A  L  R  A
                .         .         .         .         .         .
50521  CCTTCGCGCCCGGCGCCCGGCTCGTCAACGAGTACGGGCCGACCGAGACCACCGTCGGCT   50580
        F  A  P  G  A  R  L  V  N  E  Y  G  P  T  E  T  T  V  G  C
                .         .         .         .         .         .
50581  GCTGTGCCCACGACGTCGCACCGGACCCCGGCGAGGCGCCCATCCCCGTCGGTACCCCGA   50640
        C  A  H  D  V  A  P  D  P  G  E  A  P  I  P  V  G  T  P  I
                .         .         .         .         .         .
50641  TCGCGGGCCTCAGCGCGTGCGTCGTCGACGACGCGCTGCCCGCACCGCCCGGCGTGCGGG   50700
        A  G  L  S  A  C  V  V  D  D  A  L  P  A  P  P  G  V  R  G
                .         .         .         .         .         .
50701  GCGAGCTGTACATCGGCGGGACGGGCGTCACCCGCGGCTACCTGGGCCGGCCCGCGGCCA   50760
        E  L  Y  I  G  G  T  G  V  T  R  G  Y  L  G  R  P  A  A  T
                .         .         .         .         .         .
50761  CCGCCGCCGCCTACGTGCCGGACCCTGCCGCCCCCGGCGCCCGCCGCTACCGCACCGGCG   50820
        A  A  A  Y  V  P  D  P  A  A  P  G  A  R  R  Y  R  T  G  D
                .         .         .         .         .         .
50821  ACCTGGCACGCCGGCTGCCGGACGGCACCCTGCTCCTGGCGGGGCGCGCCGACCGCCAGG   50880
        L  A  R  R  L  P  D  G  T  L  L  L  A  G  R  A  D  R  Q  V
                .         .         .         .         .         .
50881  TGAAGATCCGCGGCCACCGGGTGGAACCGGGGGAGGTCGAGCAGGTGCTCGGCGGCCACC   50940
        K  I  R  G  H  R  V  E  P  G  E  V  E  Q  V  L  G  G  H  P
                .         .         .         .         .         .
50941  CCGGGGTGCGGGAGGCGGCGGTCGTCGCCCACCCGGCACCCGGCGGCGGCCGCCGGCTGG   51000
        G  V  R  E  A  A  V  V  A  H  P  A  P  G  G  G  R  R  L  V
                .         .         .         .         .         .
51001  TCGCGTACTGGGTACCGGCCGAACCGGCCCGGCCACCGTCCGCGGACGCGCTCACCGCGC   51060
        A  Y  W  V  P  A  E  P  A  R  P  P  S  A  D  A  L  T  A  L
                .         .         .         .         .         .
51061  TGCTCGCCGACCGGCTGCCGCCGTACGCGGTCCCCGCCGAACTCGTCCGCCTGCCCGCCC   51120
        L  A  D  R  L  P  P  Y  A  V  P  A  E  L  V  R  L  P  A  L
                .         .         .         .         .         .
51121  TGCCCACCACCCCCAACGGCAAGGTCGACCACACCCGGCTGCCCGCGGCCGGACGGGACC   51180
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          P   T   T   P   N   G   K   V   D   H   T   R   L   P   A   A   G   R   D   R
                      .             .             .             .             .             .
51181  GGCGACTGGCGGAACTGCTCGACCGGATCGAGGCACTGTCCGACGCCGAGGCGGCCTCGG   51240
          R   L   A   E   L   L   D   R   I   E   A   L   S   D   A   E   A   A   S   A
                      .             .             .             .             .             .
51241  CACTGCGCGACAGCCGGCCCGCACCCGGGAGTGGCGATGACCGAGCATGACGACCACCCG   51300
          L   R   D   S   R   P   A   P   G   S   G   D   D   R   A   *
                      .             .             .             .             .             .
51301  CCGGCCCGCCGGGGCCCCGCCGGTTCCGCTGGCCCCGGCGGAAGCCCGCCCGTCCCGCAC   51360
                      .             .             .             .             .             .
51361  GTGCCGGTGCCCGGGCATGACGACCGCGTCGGACGGCTGCCGGCGGACCGGAGCGTCCCG   51420
                      .             .             .             .             .             .
51421  CCGACCCGCCGATTCTCTGGGGACCCCGCCGGTTCCGGTGGTGGCCCGCCCGTCCCGCAC   51480
                      .             .             .             .             .             .
51481  CCGGAGGTGCCGATGCGCGGGCATGACGACCGCGTCGGACGGCTGTCGGCGGACTGGAGC   51540
                            M   R   G   H   D   D   R   V   G   R   L   S   A   D   W   S     (orf21)
                      .             .             .             .             .             .
51541  GTCCCGCCGACCCGCCTGCCCGCCGGGGACCCGGCCGGTTCCGTCGGCCCCGGCGGAGGC   51600
       V   P   P   T   R   L   P   A   G   D   P   A   G   S   V   G   P   G   G   G
                      .             .             .             .             .             .
51601  CCGCCCGTCCCGCACGAGGAGGTGACGATGTCGGAGTATGACGACCGCCTCGCGCGGCTG   51660
       P   P   V   P   H   E   E   V   T   M   S   E   Y   D   D   R   L   A   R   L
                      .             .             .             .             .             .
51661  TCGGACAACCAGCGCGCCCTGCTGGACCGCTGGCTCGCCGAGGACCCCGCCGGCGGTGCC   51720
       S   D   N   Q   R   A   L   L   D   R   W   L   A   E   D   P   A   G   G   A
                      .             .             .             .             .             .
51721  GGCCCGCTTCGCCCCGACGGCCGCCCGCCCCGCACCGAGGCCGAGCGGATCCTGGCCGGG   51780
       G   P   L   R   P   D   G   R   P   P   R   T   E   A   E   R   I   L   A   G
                      .             .             .             .             .             .
51781  GTCTGGGAGGAGGTGCTGGAGACCGGCGGGATCGGCGCCGACGACGACTACTTCGCGCTC   51840
       V   W   E   E   V   L   E   T   G   G   I   G   A   D   D   D   Y   F   A   L
                      .             .             .             .             .             .
51841  GGCGGAGACTCCGTCCACGCCATCGTCATCGTGGCGAAGGCCCGGCAGGCCGGACTCGCC   51900
       G   G   D   S   V   H   A   I   V   I   V   A   K   A   R   Q   A   G   L   A
                      .             .             .             .             .             .
51901  CTGACCGCCCATGACCTCTTCGAGGCCAGGACCCTCGCGGCCGTGGCGCGGAGAGCCGCC   51960
       L   T   A   H   D   L   F   E   A   R   T   L   A   A   V   A   R   R   A   A
                      .             .             .             .             .             .
51961  CCGGCCGGCCCCGCCGAGCCCGTCCCCGACGCGGGCGGCGGCGCGGTCCGGTACCCGCTG   52020
       P   A   G   P   A   E   P   V   P   D   A   G   G   G   A   V   R   Y   P   L
                      .             .             .             .             .             .
52021  ACCCCTATGCAGCAGGGCATGCTCTACCACTCGGCCGGCGGCAGCACGCCCGGCGCCTAC   52080
       T   P   M   Q   Q   G   M   L   Y   H   S   A   G   G   S   T   P   G   A   Y
                      .             .             .             .             .             .
52081  GTGGTGCAGGTGTGCTGCCGGCTGACGGGGGACCTCGACGTGGCCGCCTTCCGCACCGCC   52140
       V   V   Q   V   C   C   R   L   T   G   D   L   D   V   A   A   F   R   T   A
                      .             .             .             .             .             .
52141  TGGCAGGCCGTGCTGTCCGCCAACCCGGCGCTGGCCGTCTCCTTCCACTGGTCCGACGGC   52200
       W   Q   A   V   L   S   A   N   P   A   L   A   V   S   F   H   W   S   D   G
                      .             .             .             .             .             .
52201  TCCCCGCCCGAGCAGGTGGTGGACCCCGACGCGCGCGTCACCGTCGACACGGCCGACTGG   52260
       S   P   P   E   Q   V   V   D   P   D   A   R   V   T   V   D   T   A   D   W
                      .             .             .             .             .             .
52261  CGGGACCGCACCCCGGCCGAGCGGGACGATGCCTTCGCCCGCTTCCTGGACACCGACCGC   52320
       R   D   R   T   P   A   E   R   D   D   A   F   A   R   F   L   D   T   D   R
                      .             .             .             .             .             .
52321  GCGGCGGGCTTCGACCTCGCCCGCGCCCCGCTGATGCGGCTGACGCTCTTCCGCGAGGGC   52380
       A   A   G   F   D   L   A   R   A   P   L   M   R   L   T   L   F   R   E   G
                      .             .             .             .             .             .
52381  GAGCACGCGTACCGCTGCGTGTGGACCCACCACCACCTCGTCCTCGACGGCTGGTCCCAG   52440
       E   H   A   Y   R   C   V   W   T   H   H   H   L   V   L   D   G   W   S   Q
                      .             .             .             .             .             .
52441  CAGCTCGTCCTGCGCGACGTCCTCGACTGCTACATGCGCCTGCGCGCCGGACGCGGCGCC   52500
       Q   L   V   L   R   D   V   L   D   C   Y   M   R   L   R   A   G   R   G   A
                      .             .             .             .             .             .
52501  GAGCCGCCCGCCCGGCCGTCCTTCACCGGTCATCTGCGCCGGCTGGAGCGGCAGGACGGG   52560
       E   P   P   A   R   P   S   F   T   G   H   L   R   R   L   E   R   Q   D   G
                      .             .             .             .             .             .
52561  ATCGACGAGGAGTTCTGGCGCGACCACCTCGGCGGCCTGCCCGCACCCTCCCGCGTCGCC   52620
       I   D   E   E   F   W   R   D   H   L   G   G   L   P   A   P   S   R   V   A
                      .             .             .             .             .             .
52621  GGTCCCGGCTGCCGCGACGGCCGGGTGGTCGCCGTACGGCGCGCCGAGCACCGGCACCGG   52680
       G   P   G   C   R   D   G   R   V   V   A   V   R   R   A   E   H   R   H   R
                      .             .             .             .             .             .
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52681   GTCTCCGCGGCGACGGGCCGGGAGCTGACCGGCTTCTGCCGCCGCCACGGGCTGACCCCG   52740
        V  S  A  A  T  G  R  E  L  T  G  F  C  R  R  H  G  L  T  P
                 .         .         .         .         .         .
52741   GCCGCCGTGCTGCACGGCGGCTGGGCGGTGCTGCTGTCGCTGCACTGCGGCCAGGACGAC   52800
        A  A  V  L  H  G  G  W  A  V  L  L  S  L  H  C  G  Q  D  D
                 .         .         .         .         .         .
52801   GTGGTCTTCGGCACCACCCTCTCCGGCCGCCCCGAGGACCTGCCCGGCGTGACCGAGTGC   52860
        V  V  F  G  T  T  L  S  G  R  P  E  D  L  P  G  V  T  E  C
                 .         .         .         .         .         .
52861   GTCGGCCTCTTCATCAACACGCTTCCCCTGCGGGTCCGTTGCGGGGAGGACACGGACGTC   52920
        V  G  L  F  I  N  T  L  P  L  R  V  R  C  G  E  D  T  D  V
                 .         .         .         .         .         .
52921   GTCGACTGGCTCCACGGCGTCCAAAGCGACCTGGCCGCCCTGTGGGACCACGCGCACGTc   52980
        V  D  W  L  H  G  V  Q  S  D  L  A  A  L  W  D  H  A  H  V
                 .         .         .         .         .         .
52981   CCGCTCAGCCGCGTcGAGCGCGGTCTCGGACTGGGCCGGgGCGGCGGGCTGTTCGACAGC   53040
        P  L  S  R  V  E  R  G  L  G  L  G  R  G  G  G  L  F  D  S
                 .         .         .         .         .         .
53041   ATCATGGTCGTCGAGAACTTCCCCGCCGCCGTCGCCGACGGCCACGAGGCGGgCGGGCTG   53100
        I  M  V  V  E  N  F  P  A  A  V  A  D  G  H  E  A  G  G  L
                 .         .         .         .         .         .
53101   CGGGTGACGGAGCCCCGGGCACTCGTCGACGAGGGCTACCCCCTCGTACTGGAGGCCACC   53160
        R  V  T  E  P  R  A  L  V  D  E  G  Y  P  L  V  L  E  A  T
                 .         .         .         .         .         .
53161   ACCGGGGACCGGCCGGTGCTGCACGCCCGCTACGACCCCCACCGCCTCGCCGGCGGGCGG   53220
        T  G  D  R  P  V  L  H  A  R  Y  D  P  H  R  L  A  G  G  R
                 .         .         .         .         .         .
53221   GTCCAGGCGCTGCTCGCCGCCTTCGACGACTACCTCCGGGCGGTGACCGCCGACCCGGCC   53280
        V  Q  A  L  L  A  A  F  D  D  Y  L  R  A  V  T  A  D  P  A
                 .         .         .         .         .         .
53281   CGCCCGCTGCCGGACCTCCGCGCGGTCCTGGCCCGCGACCACGCGCGCCGGGACGGCGCG   53340
        R  P  L  P  D  L  R  A  V  L  A  R  D  H  A  R  R  D  G  A
                 .         .         .         .         .         .
53341   GCACGCGGGCGGCGCCGCGCCGCGGACCGCACCCGTCTGACGCTGGCCCGCCGCCGCCCG   53400
        A  R  G  R  R  R  A  A  D  R  T  R  L  T  L  A  R  R  R  P
                 .         .         .         .         .         .
53401   GCGACGACGACCGAGGGAGAGACACCGTGACATGGACCGTGGTGACCGGAGCCGGCGGCT   53460
        A  T  T  T  E  G  E  T  P  *
                                  M  T  W  T  V  V  T  G  A  G  G  F   (orf20)
                 .         .         .         .         .         .
53461   TCATCGGCTCCCACCTCGTACGCCGCCTCGTCCGGGACGGGCACCGGGTCCGCGGCGTGG   53520
          I  G  S  H  L  V  R  R  L  V  R  D  G  H  R  V  R  G  V  D
                 .         .         .         .         .         .
53521   ACCTGGTGCCGCCGCGCTACGGCCCCGGCGAGGCCCAGGAGTTCGTCATCGCCGACCTGC   53580
          L  V  P  P  R  Y  G  P  G  E  A  Q  E  F  V  I  A  D  L  R
                 .         .         .         .         .         .
53581   GCGACGCGGCGCAGGCCGCGCGGGCCGTCGCCGGCGCGGACTCCGTCTTCGCGCTCGCGG   53640
          D  A  A  Q  A  A  R  A  V  A  G  A  D  S  V  F  A  L  A  A
                 .         .         .         .         .         .
53641   CCAACATGGGAGGCATCGGCTGGACCCACACCGCGCCCGCCGAGATCCTCCACGACAACC   53700
          N  M  G  G  I  G  W  T  H  T  A  P  A  E  I  L  H  D  N  L
                 .         .         .         .         .         .
53701   TGCTGATCTCCACCCACACCATCGAGGCATGCCGGGCCGCCGGCGTGCGCACCACCGTCT   53760
          L  I  S  T  H  T  I  E  A  C  R  A  A  G  V  R  T  T  V  Y
                 .         .         .         .         .         .
53761   ACACCTCCTCGGCCTGCGTCTACCCCGCGTCCCTGCAGCGCGAGCCCGACGCCGCGCCGC   53820
          T  S  S  A  C  V  Y  P  A  S  L  Q  R  E  P  D  A  A  P  L
                 .         .         .         .         .         .
53821   TGGCCGAGGACCCGGTCTTCCCCGCGGAACCCGACATGGAGTACGGCTGGGAGAAGCTGA   53880
          A  E  D  P  V  F  P  A  E  P  D  M  E  Y  G  W  E  K  L  T
                 .         .         .         .         .         .
53881   CCACGGAAATCCTGTGCGGCGCCTACCGCCGCAGCCACGGCATGGACATCAAGACAGCCC   53940
          T  E  I  L  C  G  A  Y  R  R  S  H  G  M  D  I  K  T  A  R
                 .         .         .         .         .         .
53941   GGCTGCACGCCATCTACGGCCCCCTCGGCACGTACACCGGGCCCCGCGCGAAGTCCCTGT   54000
          L  H  A  I  Y  G  P  L  G  T  Y  T  G  P  R  A  K  S  L  S
                 .         .         .         .         .         .
54001   CGATGCTCTGCGACAAGGTCGCCCGGATACCCGGCGACGAGGGGGAGATAGAGGTCTGGG   54060
          M  L  C  D  K  V  A  R  I  P  G  D  E  G  E  I  E  V  W  G
                 .         .         .         .         .         .
54061   GGGACGGGACGCAGACCCGCTCCTACTGTTACGTCGACGACTGTGTCGAAGGGCTGATCC   54120
          D  G  T  Q  T  R  S  Y  C  Y  V  D  D  C  V  E  G  L  I  R
                 .         .         .         .         .         .
54121   GGCTCGCCCGCTCCGACGTGGCGGAACCGGTCAACATCGGCTCCGAGGAGCGCGTCGACA   54180
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           L  A  R  S  D  V  A  E  P  V  N  I  G  S  E  E  R  V  D  I
                   .         .         .         .         .         .
54181     TCGCGTCGCTCGTCGAGCGGATCGCCGGGGTCGCCGGGAAGAAGGTGCGCTGCGCCTTCG    54240
           A  S  L  V  E  R  I  A  G  V  A  G  K  K  V  R  C  A  F  A
                   .         .         .         .         .         .
54241     CCCCCGACCGCCCGGTCGGGCCCCGCGGGCGCGTCTCGGACAACACCCGCTGCCGCGAAC    54300
           P  D  R  P  V  G  P  R  G  R  V  S  D  N  T  R  C  R  E  L
                   .         .         .         .         .         .
54301     TGCTCGGCTGGGCACCGGAGACGTCCCTCGCGGCCGGCCTGGAGCGCACCTACCCGTGGA    54360
           L  G  W  A  P  E  T  S  L  A  A  G  L  E  R  T  Y  P  W  I
                   .         .         .         .         .         .
54361     TCGAGCGCCAGGTCCTCGCCGAGGCCGGGAGGGCCGATGCCTGAGCACCGCACACCGGTG    54420
                                                                 M    (orf19)
           E  R  Q  V  L  A  E  A  G  R  A  D  A  *
                   .         .         .         .         .         .
54421     AAGGACCTCGGCCGGCTGCTGCTCGGGCACGCCGCGCGCTTCCGGGGCCGCGAGCTGCAG    54480
          K  D  L  G  R  L  L  L  G  H  A  A  R  F  R  G  R  E  L  Q
                   .         .         .         .         .         .
54481     GACGTCGCCACCCGGGCGCTGCGGGCCTCCGGCGGGGAGAACGCCTGGGTGGTGTCCGTC    54540
          D  V  A  T  R  A  L  R  A  S  G  G  E  N  A  W  V  V  S  V
                   .         .         .         .         .         .
54541     GTCAACACCAGTCTCCGCGCCCGCCAGGCCGTGGACCACGCGCTGCGGCTCGCCCCCCGC    54600
          V  N  T  S  L  R  A  R  Q  A  V  D  H  A  L  R  L  A  P  R
                   .         .         .         .         .         .
54601     CGCGGGCTCTCCCGGCTGCGCTACCCGTTCTCCGCCGCCCACCACACGGCCACCCCGCCC    54660
          R  G  L  S  R  L  R  Y  P  F  S  A  A  H  H  T  A  T  P  P
                   .         .         .         .         .         .
54661     CGGACCCTGTCGCTGCTGTGCCCGACCCGCGAACGCGTCGGCAACGTCGAACGCTTCCTC    54720
          R  T  L  S  L  L  C  P  T  R  E  R  V  G  N  V  E  R  F  L
                   .         .         .         .         .         .
54721     GACAGCGTCGCCCGCACCGCCGCCGCGCCCGGCCGGATAGAGGCCCTCTTCTACGTCGAC    54780
          D  S  V  A  R  T  A  A  A  P  G  R  I  E  A  L  F  Y  V  D
                   .         .         .         .         .         .
54781     GACGACGACCCCCAACTCCCTGCCTACCACGAGCTGTTCGAGCACGCCCGGTGGCGCTAC    54840
          D  D  D  P  Q  L  P  A  Y  H  E  L  F  E  H  A  R  W  R  Y
                   .         .         .         .         .         .
54841     GGACGGATCGGCCGGTGCGCCCTGCACGTCGGCGCCCCCGTCGGCGTACCCCACGCCTGG    54900
          G  R  I  G  R  C  A  L  H  V  G  A  P  V  G  V  P  H  A  W
                   .         .         .         .         .         .
54901     AACCACCTGGCCCGGAACGCGGCCGGCGACGTGCTGATGATGGCCAACGACGACCAGCTC    54960
          N  H  L  A  R  N  A  A  G  D  V  L  M  M  A  N  D  D  Q  L
                   .         .         .         .         .         .
54961     TACATCGACTACGGCTGGGACACCGCCCTCGACGCCCGCGTCACCGAACTGAGCGCCCTG    55020
          Y  I  D  Y  G  W  D  T  A  L  D  A  R  V  T  E  L  S  A  L
                   .         .         .         .         .         .
55021     CACCCCGACGGCGTCCTGTGCCTGTACTTCGACGACGGCCAGTACCCCGAGGGCGGCTGC    55080
          H  P  D  G  V  L  C  L  Y  F  D  D  G  Q  Y  P  E  G  G  C
                   .         .         .         .         .         .
55081     GACTTCCCGATGGTGACACGGCCCTGGTACGGCACCCTCGGCTACTTCACCCCGACGATC    55140
          D  F  P  M  V  T  R  P  W  Y  G  T  L  G  Y  F  T  P  T  I
                   .         .         .         .         .         .
55141     TTCCAGCAGTGGGAGGTCGAGAAGTGGGTCTTCGACATCGCCGACCGGCTGCACCGGCTC    55200
          F  Q  Q  W  E  V  E  K  W  V  F  D  I  A  D  R  L  H  R  L
                   .         .         .         .         .         .
55201     TACCCCGTCCCCGGCGTCCTCGTCGAACACCGGCACTACCAGGACTACAAGGCACCCTTC    55260
          Y  P  V  P  G  V  L  V  E  H  R  H  Y  Q  D  Y  K  A  P  F
                   .         .         .         .         .         .
55261     GACGCCACCTACCAGCGGCACCGGATGACACGGGAGAAGTCCTTCGCCGACCACGCCCTG    55320
          D  A  T  Y  Q  R  H  R  M  T  R  E  K  S  F  A  D  H  A  L
                   .         .         .         .         .         .
55321     TTCCTGCGCACCGAGCCGGACCGCGAGGCGGAGACGGACAGGCTGCGGGCCGTCATCGCC    55380
          F  L  R  T  E  P  D  R  E  A  E  T  D  R  L  R  A  V  I  A
                   .         .         .         .         .         .
55381     CGGGCAGGGAACACCCCGGACGCCGACCACGCCGACCATGCCGTTCACGACGCGGAGACC    55440
          R  A  G  N  T  P  D  A  D  H  A  D  H  A  V  H  D  A  E  T
                   .         .         .         .         .         .
55441     TTCTGGTTCACCGGCCTCCTGCGCGAGTCCCACGCCAAGCTGCTCGCGGAACTCGACGAC    55500
          F  W  F  T  G  L  L  R  E  S  H  A  K  L  L  A  E  L  D  D
                   .         .         .         .         .         .
55501     GCGCCGGGCCCGGCCGCCGGAGCCGTGCTCTTCGCCGACGGCTCCTGGACCGGCGTCGCC    55560
          A  P  G  P  A  A  G  A  V  L  F  A  D  G  S  W  T  G  V  A
                   .         .         .         .         .         .
55561     TACCGCACCCACCCGCTGGCCACCGCCCTGCTCGCCTCGATCCCCGAGGCCACCCTCGAC    55620
          Y  R  T  H  P  L  A  T  A  L  L  A  S  I  P  E  A  T  L  D
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                .         .         .         .         .         .
55621  TCCGGCCGCGCCGACCTCCTCGTCGTCCCGCCCGGCGCGTCCCACCACCACCCCGACGGC   55680
       S  G  R  A  D  L  L  V  V  P  P  G  A  S  H  H  H  P  D  G
                .         .         .         .         .         .
55681  ACCGTCGACTCCGCGTTCGGCTCCGACGCCGGCCTCCGCGTCCTGTTCGGACTGCGCGTG   55740
       T  V  D  S  A  F  G  S  D  A  G  L  R  V  L  F  G  L  R  V
                .         .         .         .         .         .
55741  CCGGACGCCGCGCAACTCCGCGTCGGCGACGGCCCGGTGCCCTGGGGCAATGGCAATGC   55800
       P  D  A  A  Q  L  R  V  G  D  G  P  V  P  W  G  N  G  Q  C
                .         .         .         .         .         .
55801  CTGATCCACGACACCGCCGCACCGAGCACCCTGCGCAACGACGGCACCGAATCTCTGGCC   55860
       L  I  H  D  T  A  A  P  S  T  L  R  N  D  G  T  E  S  L  A
                .         .         .         .         .         .
55861  GCCCTCACCTTCGTGGTGCCGCGCCCGGCACCGGGGAGTGAGGCCCGTGTGCGGCATCG   55920
       A  L  T  F  V  V  P  R  P  A  P  G  E  *
                                             M  R  P  V  C  G  I  V   (orf18)
                .         .         .         .         .         .
55921  TGGCGATCCGCTCCGCCGACGGCGGACTCGACGGCGGTGAACTCACCGCGCCGATGGCCG   55980
         A  I  R  S  A  D  G  G  L  D  G  G  E  L  T  A  P  M  A  D
                .         .         .         .         .         .
55981  ACCTGCGCCCGCGCGGCCCCGACGGCGAAGGCACCTGGGTCTCGCCCACCGGCCGGGCCG   56040
         L  R  P  R  G  P  D  G  E  G  T  W  V  S  P  T  G  R  A  A
                .         .         .         .         .         .
56041  CCCTCGGCCACACCCGGCTCGCCGTGATCGCCCCCGACGCCGGACGCCAGCCGGTCGCCG   56100
         L  G  H  T  R  L  A  V  I  A  P  D  A  G  R  Q  P  V  A  G
                .         .         .         .         .         .
56101  GCCCGGACGGCACCGTCCGGCTCGTCGTCAACGGCGAGTTCTACGGCTACCGGGAGATCC   56160
         P  D  G  T  V  R  L  V  V  N  G  E  F  Y  G  Y  R  E  I  R
                .         .         .         .         .         .
56161  GCGCGGAACTGCGCGCCGcCGGCTGCCGGTTCCGCACCGGCAGCGACAGCGAGATCGCCC   56220
         A  E  L  R  A  A  G  C  R  F  R  T  G  S  D  S  E  I  A  L
                .         .         .         .         .         .
56221  TCCACCTGTACCTGCGGGACGGCCGGCGGGCACTGGAGCGGCTGCGCGGCGAGTTCGCCT   56280
         H  L  Y  L  R  D  G  R  R  A  L  E  R  L  R  G  E  F  A  F
                .         .         .         .         .         .
56281  TCGTCCTCTGGGACGAACGCCGCGCCACCCTCTTCGCCGCCCGCGACCGGTTCGGCGTCA   56340
         V  L  W  D  E  R  R  A  T  L  F  A  A  R  D  R  F  G  V  K
                .         .         .         .         .         .
56341  AACCCCTCTACTACACCGAGCGCGACGGGCGGCTCTACGTCGCCTCGACGGTCAGGGCCC   56400
         P  L  Y  Y  T  E  R  D  G  R  L  Y  V  A  S  T  V  R  A  L
                .         .         .         .         .         .
56401  TGCTCTCCTGCGGCGCCCCCGCCCGCTGGGACACCGCCGCCTTCGCCGCGCACCTGCAGC   56460
         L  S  C  G  A  P  A  R  W  D  T  A  A  F  A  A  H  L  Q  L
                .         .         .         .         .         .
56461  TCGGCCTGCCCCCCGACCGCACCCTCTTCGCCGGCATCCGGCAGCTCCCGCCCGGCTGCC   56520
         G  L  P  P  D  R  T  L  F  A  G  I  R  Q  L  P  P  G  C  H
                .         .         .         .         .         .
56521  ACCTCATCGCCGACGCCCACGGCACCCGCGTCACCCCCTACTGGGACCTCGACTACCCGC   56580
         L  I  A  D  A  H  G  T  R  V  T  P  Y  W  D  L  D  Y  P  P
                .         .         .         .         .         .
56581  CCGCCGGCGAACTCGCCGCCCGGGGAAGCCTGGACGACCACCTGGACGCGGTACGCGAAC   56640
         A  G  E  L  A  A  R  G  S  L  D  D  H  L  D  A  V  R  E  R
                .         .         .         .         .         .
56641  GGACCGACGAGGCCGTACGGTTGCGTACCGTCGCCGACGTGCCCCTCGCCTGCCACCTCA   56700
         T  D  E  A  V  R  L  R  T  V  A  D  V  P  L  A  C  H  L  S
                .         .         .         .         .         .
56701  GCGGCGGCCTGGACTCCTCCGCCGTCGCCGCCTCCGCCGCCCGCCACACCCGGCTCACCG   56760
         G  G  L  D  S  S  A  V  A  A  S  A  A  R  H  T  R  L  T  A
                .         .         .         .         .         .
56761  CCTTCACCGTCCGCTTCGACGACCCCGCCTTCGACGAGAGCGCCGTCGCCCGGCGCACCG   56820
         F  T  V  R  F  D  D  P  A  F  D  E  S  A  V  A  R  R  T  A
                .         .         .         .         .         .
56821  CCGCCCACCTGGCCATCGACCACCGCGAAGTCGCCTCGGAACGCGCCCACTTCGCGGACC   56880
         A  H  L  A  I  D  H  R  E  V  A  S  E  R  A  H  F  A  D  H
                .         .         .         .         .         .
56881  ACCTGCGGGACGTCGTCCGCGCCGGCGAGATGGTGCAGGAGAACTCGCACGGCATCGCCC   56940
         L  R  D  V  V  R  A  G  E  M  V  Q  E  N  S  H  G  I  A  R
                .         .         .         .         .         .
56941  GGTACCTGCACAGCGCGCACATCAAGAAGGCGGGATTCACCGCCGTCCTCGCCGGGGAGG   57000
         Y  L  H  S  A  H  I  K  K  A  G  F  T  A  V  L  A  G  E  G
                .         .         .         .         .         .
57001  GCGGGGACGAACTGTTCCTCGGCTACCCCCAGTTCCGCAAGGACCTGACGCTCAGCCTGT   57060
         G  D  E  L  F  L  G  Y  P  Q  F  R  K  D  L  T  L  S  L  S
                .         .         .         .         .         .
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57061   CCGCCGACGCCCGCGACAAGGCCGACCGCGGCTACGCCCGGCTGGTCGCGGCCGGGCTCC    57120
          A  D  A  R  D  K  A  D  R  G  Y  A  R  L  V  A  A  G  L  L
                 .         .         .         .         .         .
57121   TGCCGCCGTACCTGCGCACCCTCCTCGGCACCCTCGGCTTCCTGCCCTCCTGGATCGTCG    57180
          P  P  Y  L  R  T  L  L  G  T  L  G  F  L  P  S  W  I  V  D
                 .         .         .         .         .         .
57181   ACCGCCACCTGGCCGTCACCCAGCCCGTCGCCGCCCTGCTCCGCCCCGACTTCGCCGCCG    57240
          R  H  L  A  V  T  Q  P  V  A  A  L  L  R  P  D  F  A  A  E
                 .         .         .         .         .         .
57241   AACTGGCCCGCGCCGACGCCGCCGCGCCCCTGCTCGCCGCCGGCGCCGGCCTGCTCGCCG    57300
          L  A  R  A  D  A  A  A  P  L  L  A  A  G  A  G  L  L  A  G
                 .         .         .         .         .         .
57301   GGCGCGCCCCGGCGCACCAGGCCACCTACCTCTTCGCCAAGACCTGGCTGCCCGGCTACC    57360
          R  A  P  A  H  Q  A  T  Y  L  F  A  K  T  W  L  P  G  Y  L
                 .         .         .         .         .         .
57361   TGCTCGCCGCCGAACGCCTCGACGCGGCCCAGGCCGTCGAGGTGCGGCTGCCCCTCTTCG    57420
          L  A  A  E  R  L  D  A  A  Q  A  V  E  V  R  L  P  L  F  D
                 .         .         .         .         .         .
57421   ACCACCACCTCTTCGACCTCGTCCGGCACACCCCGCCGGCCTGGTACGACAAGGACGGCA    57480
          H  H  L  F  D  L  V  R  H  T  P  P  A  W  Y  D  K  D  G  T
                 .         .         .         .         .         .
57481   CCGGCAAGTACCCGCTGCGGGCCGCCATGCGCCACCGGCTGCCGCGCGAGGTGACCGAGG    57540
          G  K  Y  P  L  R  A  A  M  R  H  R  L  P  R  E  V  T  E  G
                 .         .         .         .         .         .
57541   GCCGCAAACAGGGCTTCCTCGCACCTCCGATGGCCGACGACGACACCCTCCTCGACGCCC    57600
          R  K  Q  G  F  L  A  P  P  M  A  D  D  D  T  L  L  D  A  L
                 .         .         .         .         .         .
57601   TGCGCGAACGCCTCGCCGGACCGGGCGCGGGCGACGACCCCTTCTTCGACCCGCACGCCG    57660
          R  E  R  L  A  G  P  G  A  G  D  D  P  F  F  D  P  H  A  V
                 .         .         .         .         .         .
57661   TCCGCGCCCTGCTGGACCGGCTGGCCGCCGCACCCCCGGGGCAGCGGTCCGGCGGCGAGA    57720
          R  A  L  L  D  R  L  A  A  A  P  P  G  Q  R  S  G  G  E  K
                 .         .         .         .         .         .
57721   AACTCCTCCAACTCGTCGCGAGCACCGCCGAACTGGCCGACGAGTTCGGCCTCACCACCG    57780
          L  L  Q  L  V  A  S  T  A  E  L  A  D  E  F  G  L  T  T  A
                 .         .         .         .         .         .
57781   CCCCCAGCGGGCAGAAAGGCGGCAACGGTGGCTGACCTCGATCCCGGCACGCTCTCCGAG    57840
          P  S  G  Q  K  G  G  N  G  G  *
                 .         .         .         .         .         .
57841   GCCGAGCTGACCGCCCGGATCGCCGCCCTGTCCCCCGAACGCCGGGCGGCGTTCGAGAAG    57900
                 .         .         .         .         .         .
57901   ATGCTGCACGGCGCCGCGCACCCCCGCCCCGGCATCCCGCGCCGCGGCGCCACCGCGGCA    57960
        M  L  H  G  A  A  H  P  R  P  G  I  P  R  R  G  A  T  A  A      (orf17)
                 .         .         .         .         .         .
57961   CCGGCCTCCTACGGCCAGGAACGCCTGTGGCTGCTCACCGGGCTGCTGCCCACCGCCTAC    58020
        P  A  S  Y  G  Q  E  R  L  W  L  L  T  G  L  L  P  T  A  Y
                 .         .         .         .         .         .
58021   AACTACGCCACCGCCCTGCGGCTGCGCGGCGACCTGTCCGTCCCCGCGCTGCGCGGCGCC    58080
        N  Y  A  T  A  L  R  L  R  G  D  L  S  V  P  A  L  R  G  A
                 .         .         .         .         .         .
58081   CTGCGCGGCATCGTCCGCCGCCACGAGGTGCTGCGCACCACCTTCCGGCTGGACGGCGAC    58140
        L  R  G  I  V  R  R  H  E  V  L  R  T  T  F  R  L  D  G  D
                 .         .         .         .         .         .
58141   GACCTCATCCAGGTCGTCCACCCCACGGCGGACGTCCCCGTGCGCCTGGCCGACCTCACC    58200
        D  L  I  Q  V  V  H  P  T  A  D  V  P  V  R  L  A  D  L  T
                 .         .         .         .         .         .
58201   GGACGCTCCGCCGACACCGGGCGGCTGATGCGCGAGGAGGCCCGCCGCCCCTTCGACCTG    58260
        G  R  S  A  D  T  G  R  L  M  R  E  E  A  R  R  P  F  D  L
                 .         .         .         .         .         .
58261   GAGCACGGGCCGCTGCTGCGGCTGACCCTCTTCCGGCTCGGCCCCCGCGACCACCTCGCC    58320
        E  H  G  P  L  L  R  L  T  L  F  R  L  G  P  R  D  H  L  A
                 .         .         .         .         .         .
58321   CTGCTGGCCGTCCACCACGCCGTCACCGACGGCTGGTCCAACGGCGTCCTCGTGACCGAA    58380
        L  L  A  V  H  H  A  V  T  D  G  W  S  N  G  V  L  V  T  E
                 .         .         .         .         .         .
58381   CTCGCCACCGGCTACCGGGAACTGCGCGCCGGACGCCCCGACCGGCGGCCCGCCCCGCCG    58440
        L  A  T  G  Y  R  E  L  R  A  G  R  P  D  R  R  P  A  P  P
                 .         .         .         .         .         .
58441   GTCCAGTACGGCGACTACGCGCACTGGCAGCGCGAGCGGCTGACCGGGCCCGAACTGCGG    58500
        V  Q  Y  G  D  Y  A  H  W  Q  R  E  R  L  T  G  P  E  L  R
                 .         .         .         .         .         .
58501   GCCCTGGAGGACTACTGGCGCACCGCCGTACGCGACCTGCCCAGGACGGACCTGCCCACC    58560
        A  L  E  D  Y  W  R  T  A  V  R  D  L  P  R  T  D  L  P  T
                 .         .         .         .         .         .
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58561 GACCGCCCCCGCCCCGCCGCCCGGCGCGGCGAGGGCGCCAACCACGCCCTGCTGCTCTCG   58620
      D  R  P  R  P  A  A  R  R  G  E  G  A  N  H  A  L  L  L  S
               .         .         .         .         .         .
58621 CCGGAGCTGACCGGCCGGCTCGCCGACCTGCGCCGCCGCGAGGGCGGGTCGCTGTTCATG   58680
      P  E  L  T  G  R  L  A  D  L  R  R  R  E  G  G  S  L  F  M
               .         .         .         .         .         .
58681 CTCGTGCTCTCCGCGCTCCTGGTCGTCCTGCGTGGCACCGGCGGCCGGGACCGGCTCGCC   58740
      L  V  L  S  A  L  L  V  V  L  R  G  T  G  G  R  D  R  L  A
               .         .         .         .         .         .
58741 GTCGGCACCCTCGTCGCCGGCCGCACCCGCCCCGAACTCGAGCCGCTCATCGGCTACTTC   58800
      V  G  T  L  V  A  G  R  T  R  P  E  L  E  P  L  I  G  Y  F
               .         .         .         .         .         .
58801 GTCAACGTCCTGCTGCTGCCCTTCGAGACCGGCGGCCGGACCTCCTTCGCCGAGCTGTGG   58860
      V  N  V  L  L  L  P  F  E  T  G  G  R  T  S  F  A  E  L  W
               .         .         .         .         .         .
58861 CGGCGGGTCCGCGGCCGGCTGGTGGAGGCGTACGCCCACCAGGAACTGCCGCTGGAGAAG   58920
      R  R  V  R  G  R  L  V  E  A  Y  A  H  Q  E  L  P  L  E  K
               .         .         .         .         .         .
58921 GCCCTGGAGCTGCTGCGCGCCGACGGCACCGCCCCCGCCGACCCGCCGGTCGGCGTGGTC   58980
      A  L  E  L  L  R  A  D  G  T  A  P  A  D  P  P  V  G  V  V
               .         .         .         .         .         .
58981 TGCGTCGCCCAGCAGCCCGCCCCCGCGATCACCCTGCCCGGACTCGACGCGAGCGTCGAG   59040
      C  V  A  Q  Q  P  A  P  A  I  T  L  P  G  L  D  A  S  V  E
               .         .         .         .         .         .
59041 GACGTCGACCTGGGCACCGCCCAGTTCGACCTCGTCGTCGAGGTGCGCGAACGGCCGGAA   59100
      D  V  D  L  G  T  A  Q  F  D  L  V  V  E  V  R  E  R  P  E
               .         .         .         .         .         .
59101 GGCGTGCAGATCGCCTTCCAGTACGACCGGGACCTGTTCGACGCGGCCACGGTCCGGCTC   59160
      G  V  Q  I  A  F  Q  Y  D  R  D  L  F  D  A  A  T  V  R  L
               .         .         .         .         .         .
59161 CTCGCCGACCACGTGCACGCCGTCCTCGACCAGGCCGCCGCCGACCCCACCCTGCCCTGT   59220
      L  A  D  H  V  H  A  V  L  D  Q  A  A  A  D  P  T  L  P  C
               .         .         .         .         .         .
59221 GCCGAGCTGCCCGCCCCGCCGGCCCCCGCGGCCCCGGCCCGCACGGCCGGCGCCACGACG   59280
      A  E  L  P  A  P  P  A  P  A  A  P  A  R  T  A  G  A  T  T
               .         .         .         .         .         .
59281 CTGCACGCCCTGTTCGAGTCCCGCGCCGCGAAGAGCCCCGACGCGGTCGCCCTCGTCGAC   59340
      L  H  A  L  F  E  S  R  A  A  K  S  P  D  A  V  A  L  V  D
               .         .         .         .         .         .
59341 GGCGGCCACCGCGTCACCTACCGGACCCTCAACACCCGCGCCAACCGGCTCGCCCGCCAC   59400
      G  G  H  R  V  T  Y  R  T  L  N  T  R  A  N  R  L  A  R  H
               .         .         .         .         .         .
59401 CTGCGCGCGGTCGGCGTGCGTACCGAGGACCGGGTGGCGCTGCGCCTGCCCCGCGGCACC   59460
      L  R  A  V  G  V  R  T  E  D  R  V  A  L  R  L  P  R  G  T
               .         .         .         .         .         .
59461 GACGCGGTGACCGCCACCCTCGCCGCCCTCAAGGCCGGCGCCGCGTACGTACCCCTCGAC   59520
      D  A  V  T  A  T  L  A  A  L  K  A  G  A  A  Y  V  P  L  D
               .         .         .         .         .         .
59521 CCCGCCCTCCCCGAGGAACGGCTGACCCGCGTCCTCGCCGACGCCCGCCCCGCCGTGGTC   59580
      P  A  L  P  E  E  R  L  T  R  V  L  A  D  A  R  P  A  V  V
               .         .         .         .         .         .
59581 CTCACCCCCGCGTATCTGCACGACCGGTCCGCCGAGATCACCGCCCACGCCGGCCATGAC   59640
      L  T  P  A  Y  L  H  D  R  S  A  E  I  T  A  H  A  G  H  D
               .         .         .         .         .         .
59641 CTCAACCTCCCCGTCCACCCCGACAACCTCGCCTACCTCCTCCACACCTCCGGATCCACC   59700
      L  N  L  P  V  H  P  D  N  L  A  Y  L  L  H  T  S  G  S  T
               .         .         .         .         .         .
59701 GGCACCCCCaAGGGCGTCCTCGGCAcCCACCGGGGCGCGGTCAACCGCGTCGACTGGATG   59760
      G  T  P  K  G  V  L  G  T  H  R  G  A  V  N  R  V  D  W  M
               .         .         .         .         .         .
59761 AGCACCGCGTACCCGTTCCGGACCGGCGACGTGGCCGTCGCCCGCACCGCGCCCGGCTTC   59820
      S  T  A  Y  P  F  R  T  G  D  V  A  V  A  R  T  A  P  G  F
               .         .         .         .         .         .
59821 GTCGACGCGGTCTGGGAACTCTTCGGCCCCCTGGCCGCCGGCGTCCCCCTCGTCCTCCTG   59880
      V  D  A  V  W  E  L  F  G  P  L  A  A  G  V  P  L  V  L  L
               .         .         .         .         .         .
59881 CCGACCGACGAGGCGCGCGACCCGGCCCTGCTGACGGCGGCGCTGGAACGGCACCGGGTG   59940
      P  T  D  E  A  R  D  P  A  L  L  T  A  A  L  E  R  H  R  V
               .         .         .         .         .         .
59941 AGCCGGATGGTGACGGTCCCGTCGCTGCTGACCATGCTCCTGGACGAGTCCGCCCGCGCG   60000
      S  R  M  V  T  V  P  S  L  L  T  M  L  L  D  E  S  A  R  A
               .         .         .         .         .         .
60001 ACGGACCTCGGCACCCGCCTGGCCTGCCTCCGCACCTGGATCACCAGCGGCGAGCCCCTG   60060
      T  D  L  G  T  R  L  A  C  L  R  T  W  I  T  S  G  E  P  L
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                 .         .         .         .         .         .
60061   CCGCCCGCGCTCGCCCGGCGGTTCCACGACCGCCTGCCCGGCCGCACCCTGCTGAACCTG   60120
        P  P  A  L  A  R  R  F  H  D  R  L  P  G  R  T  L  L  N  L
                 .         .         .         .         .         .
60121   TACGGCTCCTCCGAGACCGCCGCCGACGCCACCGCGGCCCGCATCGACCCGGCGCCCGGG   60180
        Y  G  S  S  E  T  A  A  D  A  T  A  A  R  I  D  P  A  P  G
                 .         .         .         .         .         .
60181   ACTGCGCTCCCGGAGCGGTCCCCGATCGGCACGCCCATCACCGGCGTCAGCGCCCTCGTC   60240
        T  A  L  P  E  R  S  P  I  G  T  P  I  T  G  V  S  A  L  V
                 .         .         .         .         .         .
60241   CGCGGCCCGGACCTGCGCCCGCTGCCCGCGCTGATGCCCGGCGAGCTGTACGCCGGGGGC   60300
        R  G  P  D  L  R  P  L  P  A  L  M  P  G  E  L  Y  A  G  G
                 .         .         .         .         .         .
60301   GCGTGCGTGGCCCGCGGCTACCACGCCCGTCCGGCCGAGACCGCCGCGGCGTTCCCGCCG   60360
        A  C  V  A  R  G  Y  H  A  R  P  A  E  T  A  A  A  F  P  P
                 .         .         .         .         .         .
60361   GATCCCGACGGCGGGCCCGGCGCCCGGATGTTCCGTACCGGTGACAGGGCCCGGCTGCGG   60420
        D  P  D  G  G  P  G  A  R  M  F  R  T  G  D  R  A  R  L  R
                 .         .         .         .         .         .
60421   GCCGACGGCCGGCTGGAACTCCTGGGCGCGTGGACCGGCAGGTGCAGATCCGCGGCCAG   60480
        A  D  G  R  L  E  L  L  G  R  V  D  R  Q  V  Q  I  R  G  Q
                 .         .         .         .         .         .
60481   CGCGCCGAGCCCGGCGAGGTCGAACACGCCCTGCTGGCCCACCCGGCCGTACGGGCCGCC   60540
        R  A  E  P  G  E  V  E  H  A  L  L  A  H  P  A  V  R  A  A
                 .         .         .         .         .         .
60541   GCCGTCACGGCGAACCCCGACGCCACCGGCCTGTGGGCGTACGTGCGGCTCGCTCCCGGC   60600
        A  V  T  A  N  P  D  A  T  G  L  W  A  Y  V  R  L  A  P  G
                 .         .         .         .         .         .
60601   CCGTTCGCCGCCGGCTCCCCCCAGACCGAGCTGACCGCCTTCCTGCGCCGCACGCTCCCT   60660
        P  F  A  A  G  S  P  Q  T  E  L  T  A  F  L  R  R  T  L  P
                 .         .         .         .         .         .
60661   GCCCACCTCGTGCCCACCGCCGTCACCGTCCTGGACGAGCTGCCGGTGACCGCGCACGGC   60720
        A  H  L  V  P  T  A  V  T  V  L  D  E  L  P  V  T  A  H  G
                 .         .         .         .         .         .
60721   AAGACCGACCACGCGCGGCTGCCCGCCCCCGACCCCCGGGCCGGGCGCCCCGCCCCGACC   60780
        K  T  D  H  A  R  L  P  A  P  D  P  R  A  G  R  P  A  P  T
                 .         .         .         .         .         .
60781   GCCCCCCGCACCCCCACCGAGCGTACGGTCGCCGACGTCTTCGCCGGGGTGCTCGGCCTG   60840
        A  P  R  T  P  T  E  R  T  V  A  D  V  F  A  G  V  L  G  L
                 .         .         .         .         .         .
60841   GAGGGGCCGGTCGGCGCGCACGACGACTTCTTCCTCCTCGGCGGGCACTCCCTCCTCGCC   60900
        E  G  P  V  G  A  H  D  D  F  F  L  L  G  G  H  S  L  L  A
                 .         .         .         .         .         .
60901   GCCCGCAGTCGCGGCGGAACTCCGCGCCCGCCGCGGCGTCCGGATCGGGCTGAGCGACGT   60960
        A  R  S  R  G  G  T  P  R  P  P  R  R  P  D  R  A  E  R  R
                 .         .         .         .         .         .
60961   CTTCGCGGCCCCCACCGTCGCCGCAGCGTCGCCGCCCGGACCGACGCCGCCCGGCCCGGC   61020
        L  R  G  P  H  R  R  R  S  V  A  A  R  T  D  A  A  R  P  G
                 .         .         .         .         .         .
61021   ACCGGCCCCGAGCACACCCCGTTCGTCACCGACCCCGGCGCCCGGCACGAGCCGTTCCCG   61080
        T  G  P  E  H  T  P  F  V  T  D  P  G  A  R  H  E  P  F  P
                 .         .         .         .         .         .
61081   CTCACCGACGTCCAGCGGGCCTACTACGTGGGACGCGAGGGCGGGTTCGCCCTCGGCGGC   61140
        L  T  D  V  Q  R  A  Y  Y  V  G  R  E  G  G  F  A  L  G  G
                 .         .         .         .         .         .
61141   GTCTCCACCCACGCCTACCTGGAGATCGAGGCCCCGCGGATCGACGTCGCACGGTTTACC   61200
        V  S  T  H  A  Y  L  E  I  E  A  P  R  I  D  V  A  R  F  T
                 .         .         .         .         .         .
61201   GGCGCGCTGCGCGGGGTGATCGCCCGGCACCCCATGCTGCGCGCCGTGATCCGTCCCGAC   61260
        G  A  L  R  G  V  I  A  R  H  P  M  L  R  A  V  I  R  P  D
                 .         .         .         .         .         .
61261   GGGCTCCAGCAGGTGCTCACCGACGTCCCCCCGTACGACGTGGCCGTGCACGACCTGCGC   61320
        G  L  Q  Q  V  L  T  D  V  P  P  Y  D  V  A  V  H  D  L  R
                 .         .         .         .         .         .
61321   GACCTGGACGAGCCCGCGCGGCAGCGCCGACGCGCCGCGCTGCGCGAGGAGATGTCCCAC   61380
        D  L  D  E  P  A  R  Q  R  R  R  A  A  L  R  E  E  M  S  H
                 .         .         .         .         .         .
61381   CAGGTGGTGCCCGCCGACCTCTGGCCCCTGTTCGACGTCCGCGTCTCCCTCGGCCCCACG   61440
        Q  V  V  P  A  D  L  W  P  L  F  D  V  R  V  S  L  G  P  T
                 .         .         .         .         .         .
61441   GACGCCCTCGTCCACGTGGGGGTGGACGCGCTGATCTGCGACGCCCACAGCTTCGGCCTC   61500
        D  A  L  V  H  V  G  V  D  A  L  I  C  D  A  H  S  F  G  L
                 .         .         .         .         .         .
61501   GTCCTGGCCGAACTCGCGGCCCGTTACGCCGACCCCGCACGCCGCTTCCCGCCCCTGACG   61560
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        V  L  A  E  L  A  A  R  Y  A  D  P  A  R  R  F  P  P  L  T
                 .         .         .         .         .         .
61561   GCGGACTTCCGGGACCACGTCCTCCATCAGGAGGCGCTCCGCGGAACCGCCGAGTACGCG   61620
        A  D  F  R  D  H  V  L  H  Q  E  A  L  R  G  T  A  E  Y  A
                 .         .         .         .         .         .
61621   GCGGCGGAGCGGTACTGGCGCGAACGCCTGCCCGAGCTGCCGCCCGGCCCCGAACTGCCC   61680
        A  A  E  R  Y  W  R  E  R  L  P  E  L  P  P  G  P  E  L  P
                 .         .         .         .         .         .
61681   CTGGCCGTCGCGCCCGAGACCCTCGGCACCCCGCGCTTCACCCGCCGCTCCGGCCGGCTG   61740
        L  A  V  A  P  E  T  L  G  T  P  R  F  T  R  R  S  G  R  L
                 .         .         .         .         .         .
61741   GACGCGGCCTCCTGGACGGCGGTCAAGGACCGGGCCCGCCGCGCCGGGCTCAGCCCCTCC   61800
        D  A  A  S  W  T  A  V  K  D  R  A  R  R  A  G  L  S  P  S
                 .         .         .         .         .         .
61801   GGCGTACTGCTGGCGGCGTTCGCCGAGGTGATCACCGCGTGGAGCGGCCGGCCGCGCTAC   61860
        G  V  L  L  A  A  F  A  E  V  I  T  A  W  S  G  R  P  R  Y
                 .         .         .         .         .         .
61861   TCGCTGATGCTGACGGTCTTCGACCGCCCGCCGCTCCACCCGGACCTCGGGCGGATCGTC   61920
        S  L  M  L  T  V  F  D  R  P  P  L  H  P  D  L  G  R  I  V
                 .         .         .         .         .         .
61921   GGCGACTTCACCTCGCTCAGCCTGCTGGAGGTCGACCACAGTCGGCCCGGCGACTTCACC   61980
        G  D  F  T  S  L  S  L  L  E  V  D  H  S  R  P  G  D  F  T
                 .         .         .         .         .         .
61981   GACAGGGCCCGCGCCCTCCAGCGCCGCCTGTGGCAGGACCTCGACCACCTGGCGGTCGGC   62040
        D  R  A  R  A  L  Q  R  R  L  W  Q  D  L  D  H  L  A  V  G
                 .         .         .         .         .         .
62041   GGCGTGACGGTGACACGGGAACGGGCGCTGCGCCACGACGCCCGACCCGGTCTGCTCACA   62100
        G  V  T  V  T  R  E  R  A  L  R  H  D  A  R  P  G  L  L  T
                 .         .         .         .         .         .
62101   CCCGTCGTCTTCACCTCCGACCTGCCTGTCGGCGAGACCGCGGCCGAGGACGCGGACGGG   62160
        P  V  V  F  T  S  D  L  P  V  G  E  T  A  A  E  D  A  D  G
                 .         .         .         .         .         .
62161   GGAGAGGGATGGGCGCTCGGAGAGCCCGTCTACGGCGTCAGCCAGACCCCGCAGGTCCAT   62220
        G  E  G  W  A  L  G  E  P  V  Y  G  V  S  Q  T  P  Q  V  H
                 .         .         .         .         .         .
62221   CTCGACCATCAAGTCGCCGAAGACCGAGGGGAGTTGGTCTTCAACTGGGACGCCGTGGAA   62280
        L  D  H  Q  V  A  E  D  R  G  E  L  V  F  N  W  D  A  V  E
                 .         .         .         .         .         .
62281   GACCTGTTCGCCCCGGGCGCCCTGGACGCCATGTTCGCCGCCTACACCGCCTCGCTGACC   62340
        D  L  F  A  P  G  A  L  D  A  M  F  A  A  Y  T  A  S  L  T
                 .         .         .         .         .         .
62341   CGCCTGGCCCGGAGCCCCGAAGCCTGGCGGCGGCCCGGCACGCCGCCGCTGCCCACCGCC   62400
        R  L  A  R  S  P  E  A  W  R  R  P  G  T  P  P  L  P  T  A
                 .         .         .         .         .         .
62401   CAGGCGGCCGTGCGCCGGCGCACCGCCGCGACCGAGGCGCCCCTGCCCGCCCGCCTGCTG   62460
        Q  A  A  V  R  R  R  T  A  A  T  E  A  P  L  P  A  R  L  L
                 .         .         .         .         .         .
62461   CACGAGGCCGTCGGCGACGCGGCCCGGCGCCACGCCGACCTGACCGCCCTGGTCGACGGC   62520
        H  E  A  V  G  D  A  A  R  R  H  A  D  L  T  A  L  V  D  G
                 .         .         .         .         .         .
62521   GACACCCGGATGACCTACCGGCGACTGACCGAGCACGCCCGGCGCGTCGGCCGCACGCTG   62580
        D  T  R  M  T  Y  R  R  L  T  E  H  A  R  R  V  G  R  T  L
                 .         .         .         .         .         .
62581   CGCCGCCTCGGCGCCCGCCCCGGCCGCCTGGTCCCGGTGGTCGCCCGCAAGGGGTGGCGG   62640
        R  R  L  G  A  R  P  G  R  L  V  P  V  V  A  R  K  G  W  R
                 .         .         .         .         .         .
62641   CAGGCCGTCGCCGCGCTGGGCGTCCTGGAGTCGGGGGCGGCGTACCTGCCCCTGGACCCC   62700
        Q  A  V  A  A  L  G  V  L  E  S  G  A  A  Y  L  P  L  D  P
                 .         .         .         .         .         .
62701   GAACTGCCCGCCGAACGGCTCGTCCACCTCGTACGGCGCGCCGAAGCCGCCCTCCTCCTC   62760
        E  L  P  A  E  R  L  V  H  L  V  R  R  A  E  A  A  L  L  L
                 .         .         .         .         .         .
62761   ACCGAACGCGCCCTGCTGGACACGCTCGCCGTCCCCGTCGGCGTCACCGTGCTCGCGGTG   62820
        T  E  R  A  L  L  D  T  L  A  V  P  V  G  V  T  V  L  A  V
                 .         .         .         .         .         .
62821   GACGACGACGCGGCCCTCGACGCCGACGGCGGCCCGCTGCAGAGCGTGCAGAACCTCACC   62880
        D  D  D  A  A  L  D  A  D  G  G  P  L  Q  S  V  Q  N  L  T
                 .         .         .         .         .         .
62881   GACCTGGCGTACACCATCTTCACCTCGGGCTCCACCGGCGAACCCAAGGGCGTCATGATC   62940
        D  L  A  Y  T  I  F  T  S  G  S  T  G  E  P  K  G  V  M  I
                 .         .         .         .         .         .
62941   GACCACCTCGGCGCGGCCAACACCCTGGAATGCGTCAACCGCCGCTTCGGCACCGGCCCC   63000
        D  H  L  G  A  A  N  T  L  E  C  V  N  R  R  F  G  T  G  P
                 .         .         .         .         .         .
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63001   GGCGACGCGGTCCTCGCCGTCTCCTCCCCGAGCTTCGACCTCGCCGTCTACGACCTGTTC   63060
        G  D  A  V  L  A  V  S  S  P  S  F  D  L  A  V  Y  D  L  F
                 .         .         .         .         .         .
63061   GGCGTGCTGGCCGCCGGCGGCACCGTGGTCGTCCCCGCCCACGACCGCCGGCGCGACCCC   63120
        G  V  L  A  A  G  G  T  V  V  V  P  A  H  D  R  R  R  D  P
                 .         .         .         .         .         .
63121   GGACACTGGGCCGAGCTGATCCGGCGCGAGCGGGTCACCCTGTGGAACTCCGTCCCCGCG   63180
        G  H  W  A  E  L  I  R  R  E  R  V  T  L  W  N  S  V  P  A
                 .         .         .         .         .         .
63181   CTGGGCACCCTGCTCACCGAGTACGCCGAGGCCCTCGCCCCCGACGCCCTGCGCACCCTG   63240
        L  G  T  L  L  T  E  Y  A  E  A  L  A  P  D  A  L  R  T  L
                 .         .         .         .         .         .
63241   CGGGCGGTGCTCCTCAGCGGCGACTGGATCCccctcggactgcccgaccGGATCCGCGCC   63300
        R  A  V  L  L  S  G  D  W  I  P  L  G  L  P  D  R  I  R  A
                 .         .         .         .         .         .
63301   CTGTCCGCCCCCGGCGCCACCGTGATGAGCCTCGGCGGCGCGACCGAAGCCTCCATCTGG   63360
        L  S  A  P  G  A  T  V  M  S  L  G  G  A  T  E  A  S  I  W
                 .         .         .         .         .         .
63361   TCGGTCTGGTACGAGATCGGGAAGGTGCACGAGGCGTGGAGCAGCATCCCCTACGGCACC   63420
        S  V  W  Y  E  I  G  K  V  H  E  A  W  S  S  I  P  Y  G  T
                 .         .         .         .         .         .
63421   CCCATGGCCAACCAGCGGCTGGAGGTCCTCGACGAGCAGCTGCGGCCCCGGCCCGACTGG   63480
        P  M  A  N  Q  R  L  E  V  L  D  E  Q  L  R  P  R  P  D  W
                 .         .         .         .         .         .
63481   GTGCCCGGCGAGCTGTACATCGGCGGCACCGGCGTCGCCAAGGGCTACTGGCGCGACCCG   63540
        V  P  G  E  L  Y  I  G  G  T  G  V  A  K  G  Y  W  R  D  P
                 .         .         .         .         .         .
63541   GAACAGACCTCCCTGCGCTTCCCCGTCCACCCGGGCAGCGGGCAACGCCTGTACCGCACC   63600
        E  Q  T  S  L  R  F  P  V  H  P  G  S  G  Q  R  L  Y  R  T
                 .         .         .         .         .         .
63601   GGGGACTTCGCCCGCCACCTCCCCGACGGCACGCTGGAATTCCTGGGCCGGCAGGACGAC   63660
        G  D  F  A  R  H  L  P  D  G  T  L  E  F  L  G  R  Q  D  D
                 .         .         .         .         .         .
63661   CAGGTGAAGATCGGCGGATTCCGGGTCGAACTGGGCGAGGTCGAGGCGGCCCTCGGCCGA   63720
        Q  V  K  I  G  G  F  R  V  E  L  G  E  V  E  A  A  L  G  R
                 .         .         .         .         .         .
63721   CTGCCCGACGTCGCCGCCGGCGCGGTGATCGCCACCGGTGACCCGCGGGGCGACCGCCGC   63780
        L  P  D  V  A  A  G  A  V  I  A  T  G  D  P  R  G  D  R  R
                 .         .         .         .         .         .
63781   CTCGTCGGCTTCGCCGTACCGGCCCGGGAGGGCGGCTTCGACGCGGCCGGGCTCCGACGG   63840
        L  V  G  F  A  V  P  A  R  E  G  G  F  D  A  A  G  L  R  R
                 .         .         .         .         .         .
63841   CAACTCGCCCGGCGGCTGCCCGCCTACATGGTCCCCACGACCCTGCTGCCCCTGGACCGG   63900
        Q  L  A  R  R  L  P  A  Y  M  V  P  T  T  L  L  P  L  D  R
                 .         .         .         .         .         .
63901   CTGCCGCTGACCGCCAACGGCAAGGTCGACCGGGCCGCACTCCAACGCCTCGTCCCCGGC   63960
        L  P  L  T  A  N  G  K  V  D  R  A  A  L  Q  R  L  V  P  G
                 .         .         .         .         .         .
63961   CGCGCACCGGCCCCGGCGGAACCCGCCACCGCCCCACCTGCCCGTTCCCGCGCCGTCCCC   64020
        R  A  P  A  P  A  E  P  A  T  A  P  P  A  R  S  R  A  V  P
                 .         .         .         .         .         .
64021   GTGCCCGGCTGGCTCGCCGACCTGTGGTGCGAACTCCTCGACGTGCCGGAGGCCGACCCC   64080
        V  P  G  W  L  A  D  L  W  C  E  L  L  D  V  P  E  A  D  P
                 .         .         .         .         .         .
64081   GACGCGAACTTCTTCGCCCTCGGCGGCACCTCCCGGGTCGCGATCACCCTGGTCACCCGG   64140
        D  A  N  F  F  A  L  G  G  T  S  R  V  A  I  T  L  V  T  R
                 .         .         .         .         .         .
64141   ATCGAGGCCCGACTCGCCGTCCGGGTGCCCCTCGCCCGCCTCTTCGACGCCCGCACCCTG   64200
        I  E  A  R  L  A  V  R  V  P  L  A  R  L  F  D  A  R  T  L
                 .         .         .         .         .         .
64201   GGCGGCCTCGCCGAGACGATCGCCGAACTGTCGGCCGCCGCCGAGGAGGAGCCGGCACCC   64260
        G  G  L  A  E  T  I  A  E  L  S  A  A  A  E  E  E  P  A  P
                 .         .         .         .         .         .
64261   GCCGAGCCCGTGTACGCCCCCGACCCCGCCACCCGCCACGAGCCGTTCCCGCTCACCGAC   64320
        A  E  P  V  Y  A  P  D  P  A  T  R  H  E  P  F  P  L  T  D
                 .         .         .         .         .         .
64321   ATCCAGCGCGCCTACTGGCTCGGCCGGCACCGCTCCCTCTCCCTTGGCGGCGTCGCCACG   64380
        I  Q  R  A  Y  W  L  G  R  H  R  S  L  S  L  G  G  V  A  T
                 .         .         .         .         .         .
64381   CACACCTACCTCGAACTCGACGTCGAGGACCTCGACCCCGGCCGGCTCCAGACGGCCCTC   64440
        H  T  Y  L  E  L  D  V  E  D  L  D  P  G  R  L  Q  T  A  L
                 .         .         .         .         .         .
64441   CGCCGGCTGATCGACCGCCACGACGCCCTCCGGCTCGTGGTCCTCCCCGACGGCCGGCAA   64500
        R  R  L  I  D  R  H  D  A  L  R  L  V  V  L  P  D  G  R  Q
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                .         .         .         .         .         .
64501  CAGATCCTCGGCGACGTACCGCCGTACCTCCTCGCCCACACCGACCTGCGGGGCAGGGCG   64560
       Q  I  L  G  D  V  P  P  Y  L  L  A  H  T  D  L  R  G  R  A
                .         .         .         .         .         .
64561  GACGCCGAGGCCGAACTGGCCCGCGTCCGCGAGCACATGTCGCACGAGGTGCGCGACGCC   64620
       D  A  E  A  E  L  A  R  V  R  E  H  M  S  H  E  V  R  D  A
                .         .         .         .         .         .
64621  TCCCGCTGGCCGCTGTTCGACGTACGGACCCACCGCCTGGACGACGTCCGCACCCGGCTG   64680
       S  R  W  P  L  F  D  V  R  T  H  R  L  D  D  V  R  T  R  L
                .         .         .         .         .         .
64681  CACCTGAGCTTGGACCTGCTCATCGCCGACGCCCACAGCGTCCACGTACTCACCGGCGAC   64740
       H  L  S  L  D  L  L  I  A  D  A  H  S  V  H  V  L  T  G  D
                .         .         .         .         .         .
64741  CTGCTCACCTTCTACGCCGACCCCGACGCGGCCCTGCCGCCCCTCGGCTGCTCCTTCCGC   64800
       L  L  T  F  Y  A  D  P  D  A  A  L  P  P  L  G  C  S  F  R
                .         .         .         .         .         .
64801  GACTACGTCCTGGCCGTCCGCGCCCACGCCGAGGGCGAGCCGCGCCGCCGCGCCCTCGAC   64860
       D  Y  V  L  A  V  R  A  H  A  E  G  E  P  R  R  R  A  L  D
                .         .         .         .         .         .
64861  CACTGGCGGGCCCGGCTGGCCGACCTGCCGGGCCCGCCCGGCCTGCCGCTGCGGTGCCGG   64920
       H  W  R  A  R  L  A  D  L  P  G  P  P  G  L  P  L  R  C  R
                .         .         .         .         .         .
64921  CCCGAGGAGCTGACCGCGCCGCGGTTCGCCCGCCTCACCACCGGACTCGGCCCCGACGCC   64980
       P  E  E  L  T  A  P  R  F  A  R  L  T  T  G  L  G  P  D  A
                .         .         .         .         .         .
64981  TGGGCACGGCTGCGGCGCGCCGCGGCGGCCGCCGAACTCACCCCGGCCGCACTGATCTGC   65040
       W  A  R  L  R  R  A  A  A  A  A  E  L  T  P  A  A  L  I  C
                .         .         .         .         .         .
65041  GCCGCCTTCTGCGACGTCCTCGCCCAGTGGAGCGACACCCCCCGCTTCACCCTCAACCTC   65100
       A  A  F  C  D  V  L  A  Q  W  S  D  T  P  R  F  T  L  N  L
                .         .         .         .         .         .
65101  ACCACCTTCCACCGCCCCGCCCTGCTCCCCGGCGTGGACGACCTCGTCGGCGACTTCACC   65160
       T  T  F  H  R  P  A  L  L  P  G  V  D  D  L  V  G  D  F  T
                .         .         .         .         .         .
65161  ACCACGACCCTGCTCGGGGTCGACGGCGAGGGGGACACCTTCCGGGACCGGGCCCGCCGA   65220
       T  T  T  L  L  G  V  D  G  E  G  D  T  F  R  D  R  A  R  R
                .         .         .         .         .         .
65221  CTCCAGGACCGCATCTGGGAGGACCTCGAACACCGCGTCGTCAGCGGCGTCGAGGTCCTG   65280
       L  Q  D  R  I  W  E  D  L  E  H  R  V  V  S  G  V  E  V  L
                .         .         .         .         .         .
65281  CGGATGCTGCGCCGCGAGCGGGGCACCCACGACGCCGTCCGGATGCCGGTCGTCTTCACC   65340
       R  M  L  R  R  E  R  G  T  H  D  A  V  R  M  P  V  V  F  T
                .         .         .         .         .         .
65341  AGCACCCTGCGGGCCGCCGGCCCCGCCCCCCGGACGGCCCCGCCCGCCTGGCGGGTACGG   65400
       S  T  L  R  A  A  G  P  A  P  R  T  A  P  P  A  W  R  V  R
                .         .         .         .         .         .
65401  CCCGGCTACGCGATCAGCCAGACCCCGCAGGTCCTGCTCGACCATCAGGTGAGCGAGAGC   65460
       P  G  Y  A  I  S  Q  T  P  Q  V  L  L  D  H  Q  V  S  E  S
                .         .         .         .         .         .
65461  GACGGCCGACTGGTCTGCACCTGGGACTACGTCGCGGACGCCTACCCGCCCGGGCTGATC   65520
       D  G  R  L  V  C  T  W  D  Y  V  A  D  A  Y  P  P  G  L  I
                .         .         .         .         .         .
65521  GAGGCCATGTTCGGGGCCTTCGAGGCGCTCCTCGCCTCGCTCGCCGGTCACGACGACGAC   65580
       E  A  M  F  G  A  F  E  A  L  L  A  S  L  A  G  H  D  D  D
                .         .         .         .         .         .
65581  GCCGGCCACGACGACGACGCCGGCCACGACGACGGCCCCGGCCACGACGACGGCCCCGGC   65640
       A  G  H  D  D  D  A  G  H  D  D  G  P  G  H  D  D  G  P  G
                .         .         .         .         .         .
65641  CACGACGACGGCCCCGGCCACGACGACGGCCCCGGCCACGACGACGGCCCCGGCCGCGAC   65700
       H  D  D  G  P  G  H  D  D  G  P  G  H  D  D  G  P  G  R  D
                .         .         .         .         .         .
65701  GACAGTGCCGATCACGGCCACAGTGCCACGCACGACGACAGCGCCGCCCGAAACGACAGA   65760
       D  S  A  D  H  G  H  S  A  T  H  D  D  S  A  A  R  N  D  R
                .         .         .         .         .         .
65761  GAGGGAGGTGGACCGGAGTGACGAGCGCCCGGCCCACGCCGACACTGCTCCCCGCCGACC   65820
       E  G  G  G  P  E  *
                        M  T  S  A  R  P  T  P  T  L  L  P  A  D  Q   (orf16)
                .         .         .         .         .         .
65821  AGCGGGAGCTGCTGCGGATGATGAACGACCGCACCGCACCCGTGCCCGCGCACACCCTCA   65880
         R  E  L  L  R  M  M  N  D  R  T  A  P  V  P  A  H  T  L  T
                .         .         .         .         .         .
65881  CCGCCCAACTGGCCGACGCCGCGCGCACGCACGACCGGGCTCTGGCACTGGTGGCACCGG   65940
         A  Q  L  A  D  A  A  R  T  H  D  R  A  L  A  L  V  A  P  G
                .         .         .         .         .         .
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65941  GTCTGACACTGAGCCACGCCGAACTGGACGCCCGGGCGGCCGCGGTGGCCGCCCGGCTCA  66000
        L  T  L  S  H  A  E  L  D  A  R  A  A  A  V  A  A  R  L  T
             .         .         .         .         .         .
66001  CCGCCGCGGGCGTCATCCCCGGGGACCGGGTCGCCCTCGCCGTCGAGTACGGCTGGGAGC  66060
        A  A  G  V  I  P  G  D  R  V  A  L  A  V  E  Y  G  W  E  Q
             .         .         .         .         .         .
66061  AGGTCGTGGGCGCCCTGGCCGCGCTCCGCGCCGGAGCCGTCTGCCTGCCCGTCGCCCCCG  66120
        V  V  G  A  L  A  A  L  R  A  G  A  V  C  L  P  V  A  P  G
             .         .         .         .         .         .
66121  GGCTGCCCCGGCCCGCCCGCTGGCAGCACGCCACCCGGGCCGGGGCGACGGCCGTCCTCA  66180
        L  P  R  P  A  R  W  Q  H  A  T  R  A  G  A  T  A  V  L  T
             .         .         .         .         .         .
66181  CCCAGTCCTGGCTCACCCAGCGCATCGACTGGCCGCAGGAACTGCCCGTCCTCTCCGTGG  66240
        Q  S  W  L  T  Q  R  I  D  W  P  Q  E  L  P  V  L  S  V  D
             .         .         .         .         .         .
66241  ACGAACCCGGGCCGCCGGTACCACCCACCACCGCCCCGGCCGACGGACGGTCCGCGACCG  66300
        E  P  G  P  P  V  P  P  T  T  A  P  A  D  G  R  S  A  T  D
             .         .         .         .         .         .
66301  ACGCCGCCTACCGGCTGGACGCCCCCGTCAGCCACCGCGCGATCACCACCGCCGCCCTGG  66360
        A  A  Y  R  L  D  A  P  V  S  H  R  A  I  T  T  A  A  L  E
             .         .         .         .         .         .
66361  AGATCGACCGCGCCTTCCGCGTCGGACCCGGCGACCGGCTCCTGGCCCTGGCCCCCGCCG  66420
        I  D  R  A  F  R  V  G  P  G  D  R  L  L  A  L  A  P  A  D
             .         .         .         .         .         .
66421  ACTCGCCGCTCGCTCTCTACGAACTGTTCGGGCCCCTCCTGGCCGGTGCGGCCCTCGTCC  66480
        S  P  L  A  L  Y  E  L  F  G  P  L  L  A  G  A  A  L  V  L
             .         .         .         .         .         .
66481  TCACCCGGGACATCGACCTGCGCGATCCCGGAGCCCTGCACGAGGCGCTGCGCACCCACG  66540
        T  R  D  I  D  L  R  D  P  G  A  L  H  E  A  L  R  T  H  G
             .         .         .         .         .         .
66541  GCGTCACCCTCTGGCACTCGCCGCCCGCCCTCCTCGGCCTCCTCCTCGACCACCTCGCCG  66600
        V  T  L  W  H  S  P  P  A  L  L  G  L  L  L  D  H  L  A  D
             .         .         .         .         .         .
66601  ACCGGGGCGGCAAACTGCCCGAGTCGCTCCGGCTGGTGCTGCTCGGCGGCGAACGCCTCG  66660
        R  G  G  K  L  P  E  S  L  R  L  V  L  L  G  G  E  R  L  D
             .         .         .         .         .         .
66661  ACCCCGCCCTCGTCCGCCGCGTCCGCGAGAGCGCCCCGCACCAGCCGGCCGTCGCCCACC  66720
        P  A  L  V  R  R  V  R  E  S  A  P  H  Q  P  A  V  A  H  L
             .         .         .         .         .         .
66721  TCTCCTCGGCCACCCCGTCCGGCCCCTGGACCACCTGCCTGGAGACCGGCGACCTCGCCC  66780
        S  S  A  T  P  S  G  P  W  T  T  C  L  E  T  G  D  L  A  P
             .         .         .         .         .         .
66781  CGGAATGGCGCTCGGTCCCCGTCGGCGCGCCCCTGCCCAACCAGCGGGCGCACATCCTGT  66840
        E  W  R  S  V  P  V  G  A  P  L  P  N  Q  R  A  H  I  L  S
             .         .         .         .         .         .
66841  CCGAGACCCTGCGGCCCTGCCCGGTCTGGGTCACCGGCCGCCTCCACTACGGCGGCGTCG  66900
        E  T  L  R  P  C  P  V  W  V  T  G  R  L  H  Y  G  G  V  A
             .         .         .         .         .         .
66901  CCGCCGAGCCCCCCACCGGAGAGGAGCACGCACCCGCGACCGTCCCGCACCCGGAGACCG  66960
        A  E  P  P  T  G  E  E  H  A  P  A  T  V  P  H  P  E  T  G
             .         .         .         .         .         .
66961  GCGAACCGCTGCTGCGCACCGGGCTGTTCGCCCGCCTGCTGCCCGAGGGCCTGATCGACG  67020
        E  P  L  L  R  T  G  L  F  A  R  L  L  P  E  G  L  I  D  V
             .         .         .         .         .         .
67021  TCGTCGGCGACGAGACCGCCCGGATCAGCGTCCGCGACCGGCCCCTGAACCTCCAGGACA  67080
        V  G  D  E  T  A  R  I  S  V  R  D  R  P  L  N  L  Q  D  T
             .         .         .         .         .         .
67081  CCGAGACCGCCCTCGCCGCCCACGAGGACGTGCACTCCGCCGTGGTCGTCCCCGTCGGGC  67140
        E  T  A  L  A  A  H  E  D  V  H  S  A  V  V  V  P  V  G  R
             .         .         .         .         .         .
67141  GGGGAGACGAGTCGCTCGCGCGGGTACGGCTCCACCCCGGCGCCACGGCCGGCCCCGACG  67200
        G  D  E  S  L  A  R  V  R  L  H  P  G  A  T  A  G  P  D  E
             .         .         .         .         .         .
67201  AACTCCTCGCCCATCTGCGCCGCAAGGTCTCCCCTTACCTGCTGCCCGGCCACATCGAGG  67260
        L  L  A  H  L  R  R  K  V  S  P  Y  L  L  P  G  H  I  E  V
             .         .         .         .         .         .
67261  TGGGCGGTCCGCTGCCGCTCACCCGGGACGGGCGCGTGGACCGCGCGCGCGTCACCGCCG  67320
        G  G  P  L  P  L  T  R  D  G  R  V  D  R  A  R  V  T  A  E
             .         .         .         .         .         .
67321  AGGCCCCCGCCCCCGCTGCCGTGCCCGCCGCCGCGCCGGCGGCGTCGGCACCCGCGCGGG  67380
        A  P  A  P  A  A  V  P  A  A  A  P  A  A  S  A  P  A  R  D
             .         .         .         .         .         .
67381  ACGAGGCCGAACTCCTCGCCCAAGTGGCCCGGGTGACCTGCCGGGTGCTGGGAATCGGCG  67440
        E  A  E  L  L  A  Q  V  A  R  V  T  C  R  V  L  G  I  G  A
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                 .         .         .         .         .         .
67441   CCGTCGAACCCGATATGAACCTGCTCGACGCCGGTGCCACCTCCGTCGAACTCGTCCGCC   67500
          V  E  P  D  M  N  L  L  D  A  G  A  T  S  V  E  L  V  R  L
                 .         .         .         .         .         .
67501   TGGCGACCGCTCTGGAGGAGGAACTCGGCCTCGACACCGACATCGAGGAACTGCTGGCCT   67560
          A  T  A  L  E  E  E  L  G  L  D  T  D  I  E  E  L  L  A  F
                 .         .         .         .         .         .
67561   TCCCGTCGGTCGCCGTGATCGTCGGCCGCCACCTCGGCCGCCGGACGGCACCACCGGCCC   67620
          P  S  V  A  V  I  V  G  R  H  L  G  R  R  T  A  P  P  A  R
                 .         .         .         .         .         .
67621   GGGACCCCCTGCCGCCCGCGTCCGTAGCGTTCGCACCCGGGTCCGTACTGCCCGCGCCGC   67680
          D  P  L  P  P  A  S  V  A  F  A  P  G  S  V  L  P  A  P  P
                 .         .         .         .         .         .
67681   CCGCGCCCGGACCCGTGCCGCCCGCGTCCGTGCCGCCCGCACCCGCGTCCGTACCGCCCG   67740
          A  P  G  P  V  P  P  A  S  V  P  P  A  P  A  S  V  P  P  A
                 .         .         .         .         .         .
67741   CGTCCGAGTCCTCACCGCTCGCGCCGCCCGCACCCGGGCCCGTGCCACCCACGCCCGTCC   67800
          S  E  S  S  P  L  A  P  P  A  P  G  P  V  P  P  T  P  V  P
                 .         .         .         .         .         .
67801   CGCCCGCCTCCGTCCCGCCCGCGTCCGGGGCCGCGCCGCACGTACCGCCCGCGCCGCCCG   67860
          P  A  S  V  P  P  A  S  G  A  A  P  H  V  P  P  A  P  P  A
                 .         .         .         .         .         .
67861   CACCCATCCCCGCGCCCTCCGTGCCccccgcgccccgcccccaaccgcccctgctcaccg   67920
          P  I  P  A  P  S  V  P  P  A  P  R  P  Q  P  P  L  L  T  G
                 .         .         .         .         .         .
67921   gcatcggcgcccgccaggcgTTCAAGGACGCCCACCACGGCATCCGGCACGAGTTCGACG   67980
          I  G  A  R  Q  A  F  K  D  A  H  H  G  I  R  H  E  F  D  A
                 .         .         .         .         .         .
67981   CCACCGACGGCGTCGCCCTCAGCGGCCCGGACGACCACCACCTCACCGCCCGTCGCAGCC   68040
          T  D  G  V  A  L  S  G  P  D  D  H  H  L  T  A  R  R  S  H
                 .         .         .         .         .         .
68041   ACCACCGCTTCGACCCCGGCCCCGTGACGCTGCCGGACCTGGCCGCCCTCCTCGGGGCCC   68100
          H  R  F  D  P  G  P  V  T  L  P  D  L  A  A  L  L  G  A  L
                 .         .         .         .         .         .
68101   TCCGCCGGGTCCGCGGCCCGGGAGGCGAACCCAAATACGCCTATCCGTCGGCCGGTTCCT   68160
          R  R  V  R  G  P  G  G  E  P  K  Y  A  Y  P  S  A  G  S  S
                 .         .         .         .         .         .
68161   CCTACCCCGTCCAGACCTACCTGCTCGTCCACCCGGGGAAGGTGACCGGACTGCCCGGCG   68220
          Y  P  V  Q  T  Y  L  L  V  H  P  G  K  V  T  G  L  P  G  G
                 .         .         .         .         .         .
68221   GCAGCCACTACGTCCACCCCGCGCGCAACCGCCTGGTGAGCATCGACCCCACCGCGACCC   68280
          S  H  Y  V  H  P  A  R  N  R  L  V  S  I  D  P  T  A  T  L
                 .         .         .         .         .         .
68281   TGCCCGCCGACGCGCACGCCGAGATCAACCGCGCCGCCTACGGGGAGGCGGCCTTCTCCC   68340
          P  A  D  A  H  A  E  I  N  R  A  A  Y  G  E  A  A  F  S  L
                 .         .         .         .         .         .
68341   TCTACCTCATCGCCGCGATCGACGCGATCACACCGCTCTACGGCGATCTCTCCTGGGACT   68400
          Y  L  I  A  A  I  D  A  I  T  P  L  Y  G  D  L  S  W  D  F
                 .         .         .         .         .         .
68401   TCACCGTCTTCGAGGCCGGTGCCATGACCCAGTTGCTGATGCGGACCGCCGTCGGCACCG   68460
          T  V  F  E  A  G  A  M  T  Q  L  L  M  R  T  A  V  G  T  G
                 .         .         .         .         .         .
68461   GCATCGGCCTGTGCCCCGTCGGCACGATGGACCCCGCGCCGCTGCGCCGCGCGTTCGCCC   68520
          I  G  L  C  P  V  G  T  M  D  P  A  P  L  R  R  A  F  A  L
                 .         .         .         .         .         .
68521   TCACCGACCGGCACCGCTTCGTCCACGCCCTCCTCGGCGGGCGGCCCCGCACGGAGGCCC   68580
          T  D  R  H  R  F  V  H  A  L  L  G  G  R  P  R  T  E  A  P
                 .         .         .         .         .         .
68581   CGTGAACCGGCACGGCCCCCTGGCGGGCCGGCGGCAGAGCGTCGACACCCGCAGCGCCGC   68640
         M  N  R  H  G  P  L  A  G  R  R  Q  S  V  D  T  R  S  A  A    (orf15)
          *
                 .         .         .         .         .         .
68641   GTGGGTGGCGCCGACGGGCACCCCGGGGCTGCCGCTGGAGGTGGCCGCCACCCGGGACGG   68700
         W  V  A  P  T  G  T  P  G  L  P  L  E  V  A  A  T  R  D  G
                 .         .         .         .         .         .
68701   CGTCGACCCGGCCGAATGGGCCCGCACCCACCTCGACACCGTCACCGGCTGGCTGCACCG   68760
         V  D  P  A  E  W  A  R  T  H  L  D  T  V  T  G  W  L  H  R
                 .         .         .         .         .         .
68761   TCACGGAGCCGTCCTGTTCCGCGGCTTCGGCGTCGGCCTCGACGGCTTCGGCGACGTCGT   68820
         H  G  A  V  L  F  R  G  F  G  V  G  L  D  G  F  G  D  V  V
                 .         .         .         .         .         .
68821   CCACGCCCTGGCCGGATCCCCCGAGGCGTACGTCGAACGGTCGTCGCCGCGCACCGCCCT   68880
         H  A  L  A  G  S  P  E  A  Y  V  E  R  S  S  P  R  T  A  L
                 .         .         .         .         .         .
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68881   CGGGCATCACCTCTACACCGCCACCGACCACCCCGCCGACCAGCCCATCCCCCGCACAA   68940
           G  H  H  L  Y  T  A  T  D  H  P  A  D  Q  P  I  P  P  H  N
                 .         .         .         .         .         .
68941   CGAGAACTCCTACCAACTCCGCTTCCCCGGACGGCTGGTCTTCGGCTGCCTCACCCCGGC   69000
           E  N  S  Y  Q  L  R  F  P  G  R  L  V  F  G  C  L  T  P  A
                 .         .         .         .         .         .
69001   CCGGACCGGCGGCGCGACCCCGCTCGCCGACACCCGGCGCGTCCTGGGCCGCCTCGACCC   69060
           R  T  G  G  A  T  P  L  A  D  T  R  R  V  L  G  R  L  D  P
                 .         .         .         .         .         .
69061   CGCCCTCGTCGCCGCCTTCGCCCGCCGCGGGGTGCTCTACCAGCGCAACTACGGCGACGG   69120
           A  L  V  A  A  F  A  R  R  G  V  L  Y  Q  R  N  Y  G  D  G
                 .         .         .         .         .         .
69121   GATCGGCATGTCCTGGCAGGACGCCTTCCAGACCCGCGACAAGGCGGCCGTCACCGCCTA   69180
           I  G  M  S  W  Q  D  A  F  Q  T  R  D  K  A  A  V  T  A  Y
                 .         .         .         .         .         .
69181   CTGCGCCGCCCGCCGCGTCGACGTCGAATGGAAACCCGACGGCGGGCTGCGGACCACCCA   69240
           C  A  A  R  R  V  D  V  E  W  K  P  D  G  G  L  R  T  T  Q
                 .         .         .         .         .         .
69241   GGTCCGCCCCGCCCTCGCCGTCCACCCGGCGACGGGGGAGCGGGTGTGGTTCAACCACGC   69300
           V  R  P  A  L  A  V  H  P  A  T  G  E  R  V  W  F  N  H  A
                 .         .         .         .         .         .
69301   CGCGTTCTTCCACGTCTCCGCCCGGCCGCCCGCGCTGCGGGACGCCCTGCTGGCCCAGTT   69360
           A  F  F  H  V  S  A  R  P  P  A  L  R  D  A  L  L  A  Q  F
                 .         .         .         .         .         .
69361   CGACGAACGCGACCTGCCGAGCCACTCCTGCTACGGCGACGGCCGGCCCATCGAACCCGC   69420
           D  E  R  D  L  P  S  H  S  C  Y  G  D  G  R  P  I  E  P  A
                 .         .         .         .         .         .
69421   CGTCATGGAGGAACTGCACCACGCCTACGCCGCCGAACTGGTGGCGCCCGCCTGGCGGGC   69480
           V  M  E  E  L  H  H  A  Y  A  A  E  L  V  A  P  A  W  R  A
                 .         .         .         .         .         .
69481   CGGCGACGTCCTCCTCGTCGACAACCTCCTCACCGCGCACGGCAGGGAACCCTTCACCGG   69540
           G  D  V  L  L  V  D  N  L  L  T  A  H  G  R  E  P  F  T  G
                 .         .         .         .         .         .
69541   CGAACGCCGCGTCGTCGTCGGCATGGCACAGCCGCTGGACTGGGACGAGGTGAGCGCGTG   69600
           E  R  R  V  V  V  G  M  A  Q  P  L  D  W  D  E  V  S  A  *
                                                                  M      (orf14)
                 .         .         .         .         .         .
69601   ACCGCCCCCGGCACACCGCTGCCCGCGACCTTCGTCCAGCGCGGCCTGTGGCCGTCCACT   69660
         T  A  P  G  T  P  L  P  A  T  F  V  Q  R  G  L  W  P  S  T
                 .         .         .         .         .         .
69661   CGCCACGCCCGCCCGGCGGAGGTCACCCACGTCCGCGCCCTGCGCCTGACCGGGGACACC   69720
         R  H  A  R  P  A  E  V  T  H  V  R  A  L  R  L  T  G  D  T
                 .         .         .         .         .         .
69721   GACACGGCGCGGCTCACCGAGGCCGTCCGGCGGGTCACCGCCGCCCTCCCCGCCCTCACC   69780
         D  T  A  R  L  T  E  A  V  R  R  V  T  A  A  L  P  A  L  T
                 .         .         .         .         .         .
69781   GCCGAACTCTCCGGCGACGAGGAACCCCGCCTGACCCTCCGGCCGGACGCCCCCGAGGTC   69840
         A  E  L  S  G  D  E  E  P  R  L  T  L  R  P  D  A  P  E  V
                 .         .         .         .         .         .
69841   ACCCCGGTCGACCTGCGCGGAGCCCCGTCCGCCGGACGCGACGCCGTCTGCGTGGCGCTG   69900
         T  P  V  D  L  R  G  A  P  S  A  G  R  D  A  V  C  V  A  L
                 .         .         .         .         .         .
69901   CTGCGCGCCGACCGGGACCACCCTCGCGCCGGACGCCACCGGGCCCGCTTCCACCTGGTG   69960
         L  R  A  D  R  D  H  P  R  A  G  R  H  R  A  R  F  H  L  V
                 .         .         .         .         .         .
69961   CGGCTCCACGACGACGAGACGGTGCTCGCGCTCACGGCCCACACCCTCCTCCTCGACACA   70020
         R  L  H  D  D  E  T  V  L  A  L  T  A  H  T  L  L  L  D  T
                 .         .         .         .         .         .
70021   CCGTCTCTCTACGCCGTGCTCGGCGCGGTCTGCCAGGCGTACGCCGGCCGCTTCCGCCCC   70080
         P  S  L  Y  A  V  L  G  A  V  C  Q  A  Y  A  G  R  F  R  P
                 .         .         .         .         .         .
70081   GAGCACTACCGCGACGCCACCACCCTGCCCGACGCGCCCCACGCCCCCCTCTCCGGTCGG   70140
         E  H  Y  R  D  A  T  T  L  P  D  A  P  H  A  P  L  S  G  R
                 .         .         .         .         .         .
70141   GCCCGGGCCTCCCGCCGGCGCTGGTGGCACCGGCGCCTGGCCGCCCTGCCCGGCCCGGCC   70200
         A  R  A  S  R  R  R  W  W  H  R  R  L  A  A  L  P  G  P  A
                 .         .         .         .         .         .
70201   CCGGCCCCCGCCGGCCCGCCCCGCGACCGGGTGACCGAAACCCACCGGCTGCGCATCCCC   70260
         P  A  P  A  G  P  P  R  D  R  V  T  E  T  H  R  L  R  I  P
                 .         .         .         .         .         .
70261   GCAGCGCGCTGGAAAGCCCTGACCGCCCTGACCGCCCTGGGCGGCCCCCTCGGCGGCAAC   70320
         A  A  R  W  K  A  L  T  A  L  T  A  L  G  G  P  L  G  G  N
                 .         .         .         .         .         .
70321   GGCTCGCTCGCCGTCATGGCCCTGGCCGCCTGGTGCCTGCGCGCCCCGGACCACCGGGGA   70380
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         G  S  L  A  V  M  A  L  A  A  W  C  L  R  A  P  D  H  R  G
                  .         .         .         .         .         .
70381   CCGGCCCGCTTCACCACCGTCGTCGACCTGCGCGACCACCTCGGACTCGGGCCCGCCGTC   70440
         P  A  R  F  T  T  V  V  D  L  R  D  H  L  G  L  G  P  A  V
                  .         .         .         .         .         .
70441   GGCCCGTTCACCGACCGCCTCGTCTTCGGCGCCGACCTCGGCGAAGCGCCGCGCCCCTCC   70500
         G  P  F  T  D  R  L  V  F  G  A  D  L  G  E  A  P  R  P  S
                  .         .         .         .         .         .
70501   TTCCGGGACGTCACGCTGCGCGCCCAGTCCGGGTTCCTGGACGCCGTCGTGCACTACCTC   70560
         F  R  D  V  T  L  R  A  Q  S  G  F  L  D  A  V  V  H  Y  L
                  .         .         .         .         .         .
70561   CCCTACGGCGACGTCGTGGAACTCgGCAGGGAACTGGGCCGCGTCACCGCGCCCCGCACC   70620
         P  Y  G  D  V  V  E  L  G  R  E  L  G  R  V  T  A  P  R  T
                  .         .         .         .         .         .
70621   GCCGCGCACTGGGACGTGGCGCTGAACTTCTGCCGCAACCCGCCCACCAGCGCCGCCACC   70680
         A  A  H  W  D  V  A  L  N  F  C  R  N  P  P  T  S  A  A  T
                  .         .         .         .         .         .
70681   CGCGGCGAACGCACCCTCGCCGAACGCGGCCTGTCCATCGAGCTGTTCCGCGAGGCCGAC   70740
         R  G  E  R  T  L  A  E  R  G  L  S  I  E  L  F  R  E  A  D
                  .         .         .         .         .         .
70741   CTGCTCGGCGCGGCCGGCACCGGTCCCGCGCACCGGTGGGACGGCACGGTGCTCGCCCTC   70800
         L  L  G  A  A  G  T  G  P  A  H  R  W  D  G  T  V  L  A  L
                  .         .         .         .         .         .
70801   TCCCTAGGCGAACTCGGCGACGACACCGTGCTGGTCCTCGACGCCGACCGCGACCACCCG   70860
         S  L  G  E  L  G  D  D  T  V  L  V  L  D  A  D  R  D  H  P
                  .         .         .         .         .         .
70861   CACCACGGAACCGCCGACCGGCTGCTCCACCGGATGGACGAAGCGCTCCTGGCGGCCGTC   70920
         H  H  G  T  A  D  R  L  L  H  R  M  D  E  A  L  L  A  A  V
                  .         .         .         .         .         .
70921   GCCGACCCGGACGCCCCCCTGCCCCCCTTGCCCGCCCCCGCGCACACCACGAGGAGCCAC   70980
         A  D  P  D  A  P  L  P  P  L  P  A  P  A  H  T  T  R  S  H
                  .         .         .         .         .         .
70981   CGATGACCACGACCCCGCGGACCGCCGCCGAGCCCACCTACCACGTGGTGGTCAACGACG   71040
           M  T  T  T  P  R  T  A  A  E  P  T  Y  H  V  V  V  N  D  E   (orf13)
        R  *
                  .         .         .         .         .         .
71041   AGGAGCAGTACTCGATCTGGCTCGCCGAACAGGAGATCCCGGCCGGCTGGCGGGCCACCG   71100
           E  Q  Y  S  I  W  L  A  E  Q  E  I  P  A  G  W  R  A  T  G
                  .         .         .         .         .         .
71101   GAACCTCCGGCACCCAGGAGGAGTGCCTGCGCCACATCGACGAGGTGTGGACCGACATGC   71160
           T  S  G  T  Q  E  E  C  L  R  H  I  D  E  V  W  T  D  M  R
                  .         .         .         .         .         .
71161   GCCCCCGCAGCCTGCGCGAGGCCATGGCCGCGGCGGAGCACGCGGAGCCCGCTCCCGCCC   71220
           P  R  S  L  R  E  A  M  A  A  A  E  H  A  E  P  A  P  A  P
                  .         .         .         .         .         .
71221   CGGCCCCGGCCGAGGAGGAGCCGAGCCTCGTCGACCGGCTCTGCGCGGGCGACCAGCCGG   71280
           A  P  A  E  E  E  P  S  L  V  D  R  L  C  A  G  D  Q  P  V
                  .         .         .         .         .         .
71281   TGGAGTCGGTCCTCCGCCCGGAGCGCACGGCCGCCGCCCTGCGGGAGGCCGTCGACCGCG   71340
           E  S  V  L  R  P  E  R  T  A  A  A  L  R  E  A  V  D  R  G
                  .         .         .         .         .         .
71341   GCTACGTCTTCGTCCGCTTCGCCGCCACCCGCGGCGGCACCGAACTCGGCGTCGCCGTCG   71400
           Y  V  F  V  R  F  A  A  T  R  G  G  T  E  L  G  V  A  V  D
                  .         .         .         .         .         .
71401   ACCCCGCGGCGACCACCATGGACGGCACCGAGCTGCGCCTGACCGGCACCCTCACCCTCG   71460
           P  A  A  T  T  M  D  G  T  E  L  R  L  T  G  T  L  T  L  D
                  .         .         .         .         .         .
71461   ACTTCGAACCGGTCCGCTGCCACGCCCGCGTCGACGTGACCACCTTCACGGGCGAGGGCC   71520
           F  E  P  V  R  C  H  A  R  V  D  V  T  T  F  T  G  E  G  R
                  .         .         .         .         .         .
71521   GCCTGGAGCGCGTGTCCGGCACCTGACCCCCGCCGGCCACCCGGCCGTGAGGCGCGGCTC   71580
           L  E  R  V  S  G  T  *
                  .         .         .         .         .         .
71581   GGGACCGGGCCGCCGACCCACCGAAGGGAGGACCCCATGACCACCCCCATGACCACCCC   71640
                                             M  T  T  P  M  T  T  P    (orf12)
                  .         .         .         .         .         .
71641   CACGACCACCCGCACCACCACCCGCACCGCCGTCTTCGCCCACCTCCGCGCCCCCGGCCT   71700
         T  T  T  R  T  T  T  R  T  A  V  F  A  H  L  R  A  P  G  L
                  .         .         .         .         .         .
71701   CGGCGACCTCCTCCAGCGCAACATCGGCCTCGCCCTCGTCCGCCGCGCCCGCCCGGCGAC   71760
         G  D  L  L  Q  R  N  I  G  L  A  L  V  R  R  A  R  P  A  T
                  .         .         .         .         .         .
71761   GGCGGTCACCCTGGTCGTCGGCGAGGACCTGGCGGCCCGCTTCGGTCCGGCACTCACCCG   71820
         A  V  T  L  V  V  G  E  D  L  A  A  R  F  G  P  A  L  T  R
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                 .         .         .         .         .         .
71821   CCACACGTACGCCACCGACGTGCTGCCCTGCCCCCAGCGGGGCGAGGCCGACCCCCGGTG   71880
         H  T  Y  A  T  D  V  L  P  C  P  Q  R  G  E  A  D  P  R  W
                 .         .         .         .         .         .
71881   GCCCGCCTTCCTGCGCACCCTGGCCGACCGCCGCTTCGCCCTCGCCGTCGTCGACCCGGA   71940
         P  A  F  L  R  T  L  A  D  R  R  F  A  L  A  V  V  D  P  D
                 .         .         .         .         .         .
71941   CAGCCAGGGCCTGCACGCCGGCCACGCCCGGGCCGCCGGCGTGCCCGAGCGGATCGGCCT   72000
         S  Q  G  L  H  A  G  H  A  R  A  A  G  V  P  E  R  I  G  L
                 .         .         .         .         .         .
72001   GCCGCAGGACCGGCCCGGAGACGAACACATCACCCATCCCATCCGCCTCCCACGTCCCCT   72060
         P  Q  D  R  P  G  D  E  H  I  T  H  P  I  R  L  P  R  P  L
                 .         .         .         .         .         .
72061   GTGGGGGACCCCGGACCTGTACGAGTACGCCACTGCCCTCGCCGCCGCGCTGGGCCTGCC   72120
         W  G  T  P  D  L  Y  E  Y  A  T  A  L  A  A  A  L  G  L  P
                 .         .         .         .         .         .
72121   CGCACCGCCGCGCCCCGGCGACGTCCTGCCGGAGCTGCCCCGCACCCGCGGCGTCCGCCC   72180
         A  P  P  R  P  G  D  V  L  P  E  L  P  R  T  R  G  V  R  P
                 .         .         .         .         .         .
72181   GCCGACGGCCGGTCTGCCCCGTCCGCTCGTCGCCGTCCACCCCGGCGGGGCACCGCACTG   72240
         P  T  A  G  L  P  R  P  L  V  A  V  H  P  G  G  A  P  H  W
                 .         .         .         .         .         .
72241   GAACAGGAGATGGCCGCTCGAGCACTACGCCCGGCTCTGCGCCCGCCTCGCGGCCGAACT   72300
         N  R  R  W  P  L  E  H  Y  A  R  L  C  A  R  L  A  A  E  L
                 .         .         .         .         .         .
72301   CTCGGCCTCCCTCTGCCTGCTGGGCGACGAAGCCGAACGCCCCGAGCTGGAACTGCTCCG   72360
         S  A  S  L  C  L  L  G  D  E  A  E  R  P  E  L  E  L  L  R
                 .         .         .         .         .         .
72361   GCACGCCGTCCTGACGCGGTCCCCGCGAGCCGTCGTCCACCTCGAGGCGGGCGCGGACCT   72420
         H  A  V  L  T  R  S  P  R  A  V  V  H  L  E  A  G  A  D  L
                 .         .         .         .         .         .
72421   CGACCGGACCGCGAACGTCCTCGCCGACGCCGACCTGCTCGTCGGCAACGACTCCTCGCT   72480
         D  R  T  A  N  V  L  A  D  A  D  L  L  V  G  N  D  S  S  L
                 .         .         .         .         .         .
72481   CGCCCACGTCGCCGCCGCCGTCCGCACCCCGTCCGTCGTCCTCTACGGCCCGACCGGCAC   72540
         A  H  V  A  A  A  V  R  T  P  S  V  V  L  Y  G  P  T  G  T
                 .         .         .         .         .         .
72541   CGAGTACCTGTGGACCAGGATCTACCCGTACCACCGCGGGGTCTCCCTGCGGTGGCCGTG   72600
         E  Y  L  W  T  R  I  Y  P  Y  H  R  G  V  S  L  R  W  P  C
                 .         .         .         .         .         .
72601   CCAGCGGCTGCGGCACGCCGCAGGCGAACTCGCCGGCCGGCGGTGCGCGCACGGCTGCGT   72660
         Q  R  L  R  H  A  A  G  E  L  A  G  R  R  C  A  H  G  C  V
                 .         .         .         .         .         .
72661   CCTGCCCTACCAGGGCCCGGCCGGCCCGTATCCGCGCTGTCTGGCCGACCTGCCGGTGGA   72720
         L  P  Y  Q  G  P  A  G  P  Y  P  R  C  L  A  D  L  P  V  D
                 .         .         .         .         .         .
72721   CAGGGTCTGGCCGGCGGTGACCGCCCGATGGGCGAGCCCCCACCCCGTGACGATCAGGAG   72780
         R  V  W  P  A  V  T  A  R  W  A  S  P  H  P  V  T  I  R  S
                 .         .         .         .         .         .
72781   TACCCCATGAGCGCCGACCCGTCCCGGGTGCGGACGATCCTCTCCGTCAACTTCAACCAC   72840
         T  P  *
               M  S  A  D  P  S  R  V  R  T  I  L  S  V  N  F  N  H     (orf11)
                 .         .         .         .         .         .
72841   GACGGCTCCGGCGTGCTGTTGCGGGAGGGCAGGATCGCCGGCTACGTCACCACCGAGCGC   72900
        D  G  S  G  V  L  L  R  E  G  R  I  A  G  Y  V  T  T  E  R
                 .         .         .         .         .         .
72901   CGCTCCCGCCTCAAGAAGCACCCGGGCCTGCGCGAGGAGGACCTCGACGAACTGCTGGAC   72960
        R  S  R  L  K  K  H  P  G  L  R  E  E  D  L  D  E  L  L  D
                 .         .         .         .         .         .
72961   CAGGCCGGGGCCGACCTCTCCGACATCGACCACGTCATGCTCTGCAACCTGCACACCATG   73020
        Q  A  G  A  D  L  S  D  I  D  H  V  M  L  C  N  L  H  T  M
                 .         .         .         .         .         .
73021   GACACACCCGACATACCCCGGCTGCACGGCTCCGACCTCAAGGAGACCTGGCTCGCGTTC   73080
        D  T  P  D  I  P  R  L  H  G  S  D  L  K  E  T  W  L  A  F
                 .         .         .         .         .         .
73081   TGGGTCAACCAGCGCAACGACGAGGTGAGCCTGCGCGGCCGCCGCATCCCCTGCACCGTC   73140
        W  V  N  Q  R  N  D  E  V  S  L  R  G  R  R  I  P  C  T  V
                 .         .         .         .         .         .
73141   AACCCGGACCACCACCTCATCCACGCCGCCACCGCCTACTACACCTCCGGCTACGACTCG   73200
        N  P  D  H  H  L  I  H  A  A  T  A  Y  Y  T  S  G  Y  D  S
                 .         .         .         .         .         .
73201   GCGATGGCCGTGGCCATCGACCCCACCGGCTGCCGCGCCTTCGCCGGCAAGGGCAGCCGC   73260
        A  M  A  V  A  I  D  P  T  G  C  R  A  F  A  G  K  G  S  R
                 .         .         .         .         .         .
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73261  CTCTACCCCCTGCGCCGCGACCTCGACGCCTGGTTCAACGCCAACATCGGCTACTGCTAC  73320
       L  Y  P  L  R  R  D  L  D  A  W  F  N  A  N  I  G  Y  C  Y
                .         .         .         .         .         .
73321  GTCGCCGACCTGATGTTCGGCTCCAGCATCGTCGGCGCCGGCAAGGTCATGGGCCTCGCC  73380
       V  A  D  L  M  F  G  S  S  I  V  G  A  G  K  V  M  G  L  A
                .         .         .         .         .         .
73381  CCCTACGGCAGACCCGCCGACGGCGCCGGCCCCGACGAGGAACCGCCCGAGACCGTGCGC  73440
       P  Y  G  R  P  A  D  G  A  G  P  D  E  E  P  P  E  T  V  R
                .         .         .         .         .         .
73441  GACTTCGCCGCCCTGGTGGCCCTGGCCGACCGGCACCCGCGCCTCGTCGACGTCGACGGC  73500
       D  F  A  A  L  V  A  L  A  D  R  H  P  R  L  V  D  V  D  G
                .         .         .         .         .         .
73501  AGGAAGCTCAACGCCACCCTCGCCCACTACATCCAGCTGGGCCTGGAACGCCAGCTGACC  73560
       R  K  L  N  A  T  L  A  H  Y  I  Q  L  G  L  E  R  Q  L  T
                .         .         .         .         .         .
73561  GCCGTCTTCGCCGAGCTCGCCCCGCTGTGCGCCCGCAACGGCATCGCACCGGACATCTGC  73620
       A  V  F  A  E  L  A  P  L  C  A  R  N  G  I  A  P  D  I  C
                .         .         .         .         .         .
73621  CTCTCCGGCGGTACCGCCCTCAACGCCATCGCCACCCAACTCGCCTTCGAGTCGACCGGC  73680
       L  S  G  G  T  A  L  N  A  I  A  T  Q  L  A  F  E  S  T  G
                .         .         .         .         .         .
73681  TTCGAGCGCATGCACCTCCACCCCGCCTGCGGCGACGACGGCACCGCGATCGGCGCGGCG  73740
       F  E  R  M  H  L  H  P  A  C  G  D  D  G  T  A  I  G  A  A
                .         .         .         .         .         .
73741  CTCTGGCACTGGCACCACGTCCTGGGCCACCCCCGGCTCCACCACACCAACGCCGACCTC  73800
       L  W  H  W  H  H  V  L  G  H  P  R  L  H  H  T  N  A  D  L
                .         .         .         .         .         .
73801  ATGTACTCCGTCCGTGAGTACCCCGAGCACACCGTCCGGCGGGCCGTGCGGGACCACGCG  73860
       M  Y  S  V  R  E  Y  P  E  H  T  V  R  R  A  V  R  D  H  A
                .         .         .         .         .         .
73861  GCCGACCTCGTCGTCGAGGAGACCGGCGACTACGTCGCCAGGGCCGCCGAACTGGTCGCC  73920
       A  D  L  V  V  E  E  T  G  D  Y  V  A  R  A  A  E  L  V  A
                .         .         .         .         .         .
73921  GGCGGCGCCGTCATCGGCTGGTACGACGGCGCCGGCGAGGTCGGGCCGCGGGCCCTGGGC  73980
       G  G  A  V  I  G  W  Y  D  G  A  G  E  V  G  P  R  A  L  G
                .         .         .         .         .         .
73981  CACCGCAGCATCGTCGCCGACCCGCGCGACCCCGCCATGCGGGACCGGCTCAACTCCCAG  74040
       H  R  S  I  V  A  D  P  R  D  P  A  M  R  D  R  L  N  S  Q
                .         .         .         .         .         .
74041  GTCAAGTTCCGCGAACACTTCCGGCCcTTCGCGCCGTCCGTGCTCAAGGAGCACGCCGCG  74100
       V  K  F  R  E  H  F  R  P  F  A  P  S  V  L  K  E  H  A  A
                .         .         .         .         .         .
74101  GAGTGGTTCGGCCTCTCCGACAGCCCCTTCATGCTGCGGGCCACCCCCGTCCTCAAGCCC  74160
       E  W  F  G  L  S  D  S  P  F  M  L  R  A  T  P  V  L  K  P
                .         .         .         .         .         .
74161  GGCGTGCCCGCCATCACCCACGTCGACGGGACGTCGAGGATCCAGTCGGTCACCCGCCAG  74220
       G  V  P  A  I  T  H  V  D  G  T  S  R  I  Q  S  V  T  R  Q
                .         .         .         .         .         .
74221  GACACCCCCGCCTTCCACGACCTCATCCACGCCTTCAAGGACCGTACGGGGATCCCCATG  74280
       D  T  P  A  F  H  D  L  I  H  A  F  K  D  R  T  G  I  P  M
                .         .         .         .         .         .
74281  GTGCTCAACACCAGCCTCAACACCAAGGGCGAGCCGATCGCGGAGACACCCGAGGACGCC  74340
       V  L  N  T  S  L  N  T  K  G  E  P  I  A  E  T  P  E  D  A
                .         .         .         .         .         .
74341  CTGCGCACCCTGCTCGGCTCCCGGCTCGACCACCTGGTGCTCCCGGGCCTCATCGTCAGC  74400
       L  R  T  L  L  G  S  R  L  D  H  L  V  L  P  G  L  I  V  S
                .         .         .         .         .         .
74401  GGCCGGACGGCGGCCCGCTCATGAGCGCCCCGCGGGGCGAGCGGACCCGGCGCCGCGCGC  74460
                           M  S  A  P  R  G  E  R  T  R  R  R  A  L  (orf10)
       G  R  T  A  A  R  S  *
                .         .         .         .         .         .
74461  TCGAACGCGACATCGCCGCGATCTGGGCCGAGACCCTCGGCAGGGACAGCGTCGGCCCGC  74520
         E  R  D  I  A  A  I  W  A  E  T  L  G  R  D  S  V  G  P  H
                .         .         .         .         .         .
74521  ACGAGGACTTCGCCGCGCTGGGCGGCAACTCCATCCACGCCATCAAGATCACCAACCGGG  74580
         E  D  F  A  A  L  G  G  N  S  I  H  A  I  K  I  T  N  R  V
                .         .         .         .         .         .
74581  TGGAGGAACTCGTCGACGCCGAGCTGTCCATCCGCGTCCTGCTCGAGACGCGCACCGTGG  74640
         E  E  L  V  D  A  E  L  S  I  R  V  L  L  E  T  R  T  V  A
                .         .         .         .         .         .
74641  CCGGCATGACGGACCACGTCCACGCCACGCTCACGGGGGAGCGGGACCGGTGAACACCGA  74700
         G  M  T  D  H  V  H  A  T  L  T  G  E  R  D  R  *
                                                        M  N  T  D   (orf9)
                .         .         .         .         .         .
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```
74701  CCTGCCCCGGCTGCTCGACCGGATCGCCGGCCTGCGCGTCCTCGTCATCGGCGACGTCAT   74760
        L  P  R  L  L  D  R  I  A  G  L  R  V  L  V  I  G  D  V  I
                .         .         .         .         .         .
74761  CCTCGACACCTACGTCTGGGGAGCCACCTCGGGCCTGTGCCGCGAATCCCCCGTCCCTGC   74820
        L  D  T  Y  V  W  G  A  T  S  G  L  C  R  E  S  P  V  P  A
                .         .         .         .         .         .
74821  CGTCACCCTGACCTCCGTCGCCCACCAGTGCGGCGGCGCCGCCAACGTCGCCGTGAACCT   74880
        V  T  L  T  S  V  A  H  Q  C  G  G  A  A  N  V  A  V  N  L
                .         .         .         .         .         .
74881  CCGGGCGCTCGGCGCCGAACCGGTGCTGCTCTCCGCGACGGGTGACGACCGCGCCGGCCG   74940
        R  A  L  G  A  E  P  V  L  L  S  A  T  G  D  D  R  A  G  R
                .         .         .         .         .         .
74941  CCGGCTGCGCGAAGCCCTCCGTGCGCGGGACGTCGACACCGGCGGACTCTTCGTACAGCC   75000
        R  L  R  E  A  L  R  A  R  D  V  D  T  G  G  L  F  V  Q  P
                .         .         .         .         .         .
75001  CGGCCGGACCACGGTCACCAAACGCCGCGTCATGGCCGACGGACAGATGCTGCTCCGCCT   75060
        G  R  T  T  V  T  K  R  R  V  M  A  D  G  Q  M  L  L  R  L
                .         .         .         .         .         .
75061  CGACGAGGGCGGCGAACACCCGTTGCCCGTGGCGACGGACACCGGAAGCCGCCTGCTCGA   75120
        D  E  G  G  E  H  P  L  P  V  A  T  D  T  G  S  R  L  L  E
                .         .         .         .         .         .
75121  ACGGGCCGCCGGCCTGCTGCCCGCCGTCGACGCCGTGATCGTCTCCGACTACGGGTACGG   75180
        R  A  A  G  L  L  P  A  V  D  A  V  I  V  S  D  Y  G  Y  G
                .         .         .         .         .         .
75181  CGTGTGGGAGCCCGACACCGTCGCCCGGCTCGCCGCACACCGCGAACTCGGCCCGTCCAC   75240
        V  W  E  P  D  T  V  A  R  L  A  A  H  R  E  L  G  P  S  T
                .         .         .         .         .         .
75241  CCTGGTCGTCGACTCCCGCCGGCCCGCGCGCTTCACCGCGCTGCGGGCCAGCGCCGTCAA   75300
        L  V  V  D  S  R  R  P  A  R  F  T  A  L  R  A  S  A  V  K
                .         .         .         .         .         .
75301  ACCCAACCACGCGGAGGCGCTGCGCCTGCTCGACGCCGGCGAACCCCCGCCCGGCCCGGC   75360
        P  N  H  A  E  A  L  R  L  L  D  A  G  E  P  P  P  G  P  A
                .         .         .         .         .         .
75361  CAGGGCGGACTGGGCGGCCGCCCTCGGCGACCGGCTCCTGCGCCTGACGGGAGCCGAACG   75420
        R  A  D  W  A  A  A  L  G  D  R  L  L  R  L  T  G  A  E  R
                .         .         .         .         .         .
75421  GGTCGCCCTCACCCTGGACGCCGACGGATCACTGCTCTTCGAACGCGACCGGCCCCCGGT   75480
        V  A  L  T  L  D  A  D  G  S  L  L  F  E  R  D  R  P  P  V
                .         .         .         .         .         .
75481  CCGCACGTTCGCCCGGGGCAGCCGGGCACCGGTCACGGCCGCCGTCGGCGCCGGCGACGC   75540
        R  T  F  A  R  G  S  R  A  P  V  T  A  A  V  G  A  G  D  A
                .         .         .         .         .         .
75541  CTTCACCGCGGCCCTCACCCTCGCCCTCGCCGCCGGCGCCGACTCCGCGGTCGCCGCCGA   75600
        F  T  A  A  L  T  L  A  L  A  A  G  A  D  S  A  V  A  A  E
                .         .         .         .         .         .
75601  ACTGGCCTCCGCCGCCGCCGGCACGGCCGTCGCCACCCCCGGCACCAGCACCTGGCACGC   75660
        L  A  S  A  A  A  G  T  A  V  A  T  P  G  T  S  T  W  H  A
                .         .         .         .         .         .
75661  CGACGAACTGCGCCGACTGCTCGGCGGCACCGGCAAGGTCTGCCGGACCGGCACCCTGCC   75720
        D  E  L  R  R  L  L  G  G  T  G  K  V  C  R  T  G  T  L  P
                .         .         .         .         .         .
75721  CGCCCGGCTGCTCGACCCGGCCGCCCGCGACCGCCGGGTCGTCTTCACCAACGGCTGCTT   75780
        A  R  L  L  D  P  A  A  R  D  R  R  V  V  F  T  N  G  C  F
                .         .         .         .         .         .
75781  CGACCTCCTGCACGGCGGCCACGTCTCCTGCCTGAGCCGGGCCAAGGAACTGGGCGACCT   75840
        D  L  L  H  G  G  H  V  S  C  L  S  R  A  K  E  L  G  D  L
                .         .         .         .         .         .
75841  GCTCGTCGTCGGCGTCAACTCCGACGCGAGCGTCCGACGCCTCAAGGGCCCCCGTCGCCC   75900
        L  V  V  G  V  N  S  D  A  S  V  R  R  L  K  G  P  R  R  P
                .         .         .         .         .         .
75901  GGTGATCCCCCTCGCCGAACGCATGCGCGTCCTCGCCGCCCTGAGCTGCGTGGACCTCGT   75960
        V  I  P  L  A  E  R  M  R  V  L  A  A  L  S  C  V  D  L  V
                .         .         .         .         .         .
75961  CGTGCCCTTCGACGACGACAGCCCCGCCGCCCTCATCGAGGCCCTCCGCCCCGAGGTCTA   76020
        V  P  F  D  D  D  S  P  A  A  L  I  E  A  L  R  P  E  V  Y
                .         .         .         .         .         .
76021  CGCCAAGGGCGGGGACTACACCCTCGCGACCCTGCCCGAAGCACCCCTCGTCCAACGGCT   76080
        A  K  G  G  D  Y  T  L  A  T  L  P  E  A  P  L  V  Q  R  L
                .         .         .         .         .         .
76081  CGGCGGCGTCGTCCACCTGCTCCCCAGCGTCGCCGACACCTCCACCACCGACATCATCCG   76140
        G  G  V  V  H  L  L  P  S  V  A  D  T  S  T  T  D  I  I  R
                .         .         .         .         .         .
76141  GCGCATCCACGCCCTGTCCAGGACCGGCGAGGGAGACACCCCATGAGCCACGCCATCGGA   76200
                                                  M  S  H  A  I  G    (orf8)
        R  I  H  A  L  S  R  T  G  E  G  D  T  P  *
                 .         .         .         .         .         .
76201   CCGAGCCGGCTGATCCCCGCCATCCGCGAAGCGCTCGGGGACGAGAAGGACCCCCGGCTC   76260
        P  S  R  L  I  P  A  I  R  E  A  L  G  D  E  K  D  P  R  L
                 .         .         .         .         .         .
76261   GCCCTCTACGTCCACGTCCCCTTCTGCTCCTCCAAGTGCCACTTCTGCGACTGGGTCACC   76320
        A  L  Y  V  H  V  P  F  C  S  S  K  C  H  F  C  D  W  V  T
                 .         .         .         .         .         .
76321   GACATCCCCGTCGCACGCCTGCGCGGCGACAGCCGGGAACGCTCGCCCTACGTCACCGCC   76380
        D  I  P  V  A  R  L  R  G  D  S  R  E  R  S  P  Y  V  T  A
                 .         .         .         .         .         .
76381   CTCTGCGACCAGATCCGCTTCTACGGCCCCCAGCTCACCCGGCTCGGCTACCGCCCCGAG   76440
        L  C  D  Q  I  R  F  Y  G  P  Q  L  T  R  L  G  Y  R  P  E
                 .         .         .         .         .         .
76441   GTCATGTACTGGGGCGGCGGCACCCCCACCCGGCTCACCGGCGACGAGATGACGGCCGTC   76500
        V  M  Y  W  G  G  G  T  P  T  R  L  T  G  D  E  M  T  A  V
                 .         .         .         .         .         .
76501   CACCAGGCCCTCGACGACGCCTTCGACCTGACGGGACTCCGCCAGTGGTCGGTGGAGAGC   76560
        H  Q  A  L  D  D  A  F  D  L  T  G  L  R  Q  W  S  V  E  S
                 .         .         .         .         .         .
76561   ACCCCGAACGACCTCGACCCCGCCACCCTCGACACCCTGCGCGGCCTCGGCGTCACCCGC   76620
        T  P  N  D  L  D  P  A  T  L  D  T  L  R  G  L  G  V  T  R
                 .         .         .         .         .         .
76621   GTCAGCGTCGGCGTCCAGTCGCTCAACCCGTACCAGCTGCGCAAGGCAGGCCGGGCCCAC   76680
        V  S  V  G  V  Q  S  L  N  P  Y  Q  L  R  K  A  G  R  A  H
                 .         .         .         .         .         .
76681   TCGCGCGAACAGGCCCTGGCCGCCGTCCCCCTGTTGCGCCGCGCCGGCATCGACGAGTTC   76740
        S  R  E  Q  A  L  A  A  V  P  L  L  R  R  A  G  I  D  E  F
                 .         .         .         .         .         .
76741   AACGTCGACCTGATCGCCGGCTTCCCCGGCGAAGCCGTCGAGTCCTTCGAGGAGACCCTG   76800
        N  V  D  L  I  A  G  F  P  G  E  A  V  E  S  F  E  E  T  L
                 .         .         .         .         .         .
76801   CGCACCGTCCTCGCGCTCGACCCGCCGCACGTCTCCGTCTACCCCTACCGCGCCACCCCC   76860
        R  T  V  L  A  L  D  P  P  H  V  S  V  Y  P  Y  R  A  T  P
                 .         .         .         .         .         .
76861   AAGACGGTCATGGCCATGCAGCTCGACCGCGAGTTCGTCGAGGCCCGGAACCGGGACGGC   76920
        K  T  V  M  A  M  Q  L  D  R  E  F  V  E  A  R  N  R  D  G
                 .         .         .         .         .         .
76921   ATGATCGACGCCTATGAACGGGCCATGGCCGCGCTCGGCGCCGCCGGCTATCACGAGTAC   76980
        M  I  D  A  Y  E  R  A  M  A  A  L  G  A  A  G  Y  H  E  Y
                 .         .         .         .         .         .
76981   TGCCACGGCTACTGGGTGCGCGACGCGCGCCACGAGGACCAGGACGGCAACTACAAGTAC   77040
        C  H  G  Y  W  V  R  D  A  R  H  E  D  Q  D  G  N  Y  K  Y
                 .         .         .         .         .         .
77041   GACCTGGCCGGCGACAAGATCGGCTTTGGCAGCGGCGCCGAATCGATCATCGGTCACCAC   77100
        D  L  A  G  D  K  I  G  F  G  S  G  A  E  S  I  I  G  H  H
                 .         .         .         .         .         .
77101   CTGCTCTGGAACGAGAACAGCGCCTACGCCCGCTACCTGCTCGCCCCCCGCGAGTTCTCC   77160
        L  L  W  N  E  N  S  A  Y  A  R  Y  L  L  A  P  R  E  F  S
                 .         .         .         .         .         .
77161   GCCGCCCACCGGTTCACCACCGCCGAACCCGACCGCCTGACCGCCCCCGTCGGCGGCGCG   77220
        A  A  H  R  F  T  T  A  E  P  D  R  L  T  A  P  V  G  G  A
                 .         .         .         .         .         .
77221   CTGATGACCCGTGAAGGCGTGGTCTTCGCCCGCTTCCGCAGACTGACCGGCCTGGACTTC   77280
        L  M  T  R  E  G  V  V  F  A  R  F  R  R  L  T  G  L  D  F
                 .         .         .         .         .         .
77281   GCGGACGTCCGCGCCACACCGTACTTCCGCCAGTGGTTCGAGCTCCTGGAGCGCTGCGGC   77340
        A  D  V  R  A  T  P  Y  F  R  Q  W  F  E  L  L  E  R  C  G
                 .         .         .         .         .         .
77341   GGCCGCTTCGTCGAGACGCCGTACAGCCTCCGCCTGGAGCCGTCCACCATCCACCGCGCC   77400
        G  R  F  V  E  T  P  Y  S  L  R  L  E  P  S  T  I  H  R  A
                 .         .         .         .         .
77401   TACATCACCCACCTCGCCTACACCATGGCCCATGGCCTGGCCCCCGAACGCGCCTGA      77457
        Y  I  T  H  L  A  Y  T  M  A  H  G  L  A  P  E  R  A  *
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SEQ ID NO: 2 ORFS BLM gene cluster ORFs 31-40

(notice this part is on the reverse strand and the last nucleotide (18660) is the first (1) on the whole cluster of 77457 bp. Also the last orf (40) is incomplete and contains frame shifts)

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                 .         .         .         .         .         .
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   1  GTGACCGAGAACCTTCCGTCGTGCCCCGAATGCTCCAGCGCGTACACCTATGAGATGGGT   60
      M  T  E  N  L  P  S  C  P  E  C  S  S  A  Y  T  Y  E  M  G
      (orf31)
               .         .         .         .         .         .
  61  GCGCTCCTGGTCTGCCCCGAATGCGGCCACGAGTGGCCGCCCGCGACCGCCGAGTCCGCG   120
      A  L  L  V  C  P  E  C  G  H  E  W  P  P  A  T  A  E  S  A
               .         .         .         .         .         .
 121  GACAACCCCGAAGACGGCGCGATCAGGGACGCGGTCGGCAACGTACTCGCCGACGGCGAC   180
      D  N  P  E  D  G  A  I  R  D  A  V  G  N  V  L  A  D  G  D
               .         .         .         .         .         .
 181  ACCGTCACGGTGGTCAAGAGCCTGAAGGTCAAGGGCCACCCGACCGGCATCAAGGCCGGC   240
      T  V  T  V  V  K  S  L  K  V  K  G  H  P  T  G  I  K  A  G
               .         .         .         .         .         .
 241  ACCAAGGTGCGCAACATCCGCCTCGTGGAGGGTGTGGCCGGCCACGACATCGACTGCAAG   300
      T  K  V  R  N  I  R  L  V  E  G  V  A  G  H  D  I  D  C  K
               .         .         .         .         .         .
 301  ATCGACGGGTTCGGCGCCATGCAGCTCAAGTCCAGCGTGGTCAAGAAGGTCTGACCGGTT   360
      I  D  G  F  G  A  M  Q  L  K  S  S  V  V  K  K  V  *
               .         .         .         .         .         .
 361  ACGCCGGCCCAGGCCCTGCCCAGGCTCCACTACGCCGCGGCGCAACCGAGCCGGAACGGG   420
               .         .         .         .         .         .
 421  GCCCGGGCCCGCTCCAAGTCCCGTTCCGTGCGCGGCCGCGGCAGCCAGGCCGTGTTCACC   480
               .         .         .         .         .         .
 481  CTGGGGTCGCCGTCCCCGTTCGCACGCGTCGTACACGCCACCACGCACGGCACGGAACTC   540
               .         .         .         .         .         .
 541  CCCGAACTCGCCACGTTCCCCAAGTCCCCGCGTGCCCGGATCCGCCCGGACCGGCGTCGG   600
               .         .         .         .         .         .
 601  TCCGCCCGCCGGGCCGCGGCCGGGTCCCCGGGCCGCGGCGGGAGGGGGTCTCGCGCCGTG   660
               .         .         .         .         .         .
 661  GAACGCCGGCCGGAAATTTACGTATAGGTAGAGATCCCGGCGAAGCGATCGGCGCGTTAT   720
               .         .         .         .         .         .
 721  GGCAGCATCCGCGCCGGCCCGCCGCGCAGTTCCTCGGTCCCGGACCGATGGCGTCAAAAG   780
               .         .         .         .         .         .
 781  TGAGCGACGAAATCGCCGGATCGCGCGAGGACCGTCGCGGGCCGCACGAGGACAACCGGG   840
               .         .         .         .         .         .
 841  GGATATATCAGCGCATTCCCAGGTCACGCGTTGACTGGAAATCGCCTACTTATCGCGTCA   900
               .         .         .         .         .         .
 901  CGCCTGTAGGGATCATGGCCGGGAATGGCCTCAGACGCTTTGAGTGCCCACCTTGAGGTT   960
                              M  A  S  D  A  L  S  A  H  L  E  V
                              (orf32)
               .         .         .         .         .         .
 961  TCCGACTGTCGGCAGCGCGGGGGGATCACGGTGACGAATGACGGATCTGAACTCGCCGGG   1020
      S  D  C  R  Q  R  G  G  I  T  V  T  N  D  G  S  E  L  A  G
               .         .         .         .         .         .
1021  CAAAACGTGGCGGCGGTCCGCTTCGAGCGGTATTCCGCGATCGCGCCGGAGCGGACCGCC   1080
      Q  N  V  A  A  V  R  F  E  R  Y  S  A  I  A  P  E  R  T  A
               .         .         .         .         .         .
1081  ATCCTGCACAAAGGTGCCGCGACCGGTTACGACGAGCTCAACCGCCGGGCCGAGCTGACA   1140
      I  L  H  K  G  A  A  T  G  Y  D  E  L  N  R  R  A  E  L  T
               .         .         .         .         .         .
1141  GCCACGCGCCTGGCGGACGCGGGCGCCGGCCCCTCGACCCTGGTGGCAGTGGCCCTCCCA   1200
      A  T  R  L  A  D  A  G  A  G  P  S  T  L  V  A  V  A  L  P
               .         .         .         .         .         .
1201  CGCGATCCCGACCTCGTCGCCACCCTGTGCGCCCTGCTCAAACTGGGTGCCGCATGCCTT   1260
      R  D  P  D  L  V  A  T  L  C  A  L  L  K  L  G  A  A  C  L
               .         .         .         .         .         .
1261  CCCCTGGATCCCGGCATACCGGCCGGGCGGCTGCGCGAGATCATGGCCGACGCGTCCCCC   1320
      P  L  D  P  G  I  P  A  G  R  L  R  E  I  M  A  D  A  S  P
               .         .         .         .         .         .
1321  GACGTTCTCGTCACCACCCGTGCCGTCGCTCCGGCATTCACCGGTGACGGACCCGTCCTT   1380
      D  V  L  V  T  T  R  A  V  A  P  A  F  T  G  D  G  P  V  L
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               .         .         .         .         .         .
1381  TTCCTGGACGACGCTCCTCCGACCTGCTCCGCCGTCCTTCCACGGCACTCAGCGGGGACC  1440
      F  L  D  D  A  P  P  T  C  S  A  V  L  P  R  H  S  A  G  T

               .         .         .         .         .         .
1441  GCGTCGGAAATCGCCTATGTGCTGTACCCGACGACTCCTGACGAGAAGTCCGAAAATTCG  1500
      A  S  E  I  A  Y  V  L  Y  P  T  T  P  D  E  K  S  E  N  S

               .         .         .         .         .         .
1501  GTCGTCTCCTATCGTGATATGGCGCGCTACCTTGACGACCCCACTGCCGGGATTCCGGCG  1560
      V  V  S  Y  R  D  M  A  R  Y  L  D  D  P  T  A  G  I  P  A

               .         .         .         .         .         .
1561  AGGGCGGAGATTCTCCGGCTGGTCGCGCCGCTCCTGTCCGGCGGTCGTCTGGTGCTGGAC  1620
      R  A  E  I  L  R  L  V  A  P  L  L  S  G  G  R  L  V  L  D

               .         .         .         .         .         .
1621  GCCGACGAGACCCGGCCCCGGCCGGTCACCCGTGAGGCGCCGCGCGACATGGTGGAGGAC  1680
      A  D  E  T  R  P  R  P  V  T  R  E  A  P  R  D  M  V  E  D

               .         .         .         .         .         .
1681  GTCGTGGCGCAGGTCTGGTGCGCCGTGCTCGGCGTGGACCGGGTGGGCGTGCGGGACCGC  1740
      V  V  A  Q  V  W  C  A  V  L  G  V  D  R  V  G  V  R  D  R

               .         .         .         .         .         .
1741  TTCTTCGACCTGGGCGGCAAGTCGCTGGCGGCGGTCCAGGTGGTGGCGCGCCTGCGGAAG  1800
      F  F  D  L  G  G  K  S  L  A  A  V  Q  V  V  A  R  L  R  K

               .         .         .         .         .         .
1801  CTGCTCGGCGTCGAGCTGCCGCTGCGGGCCCTGTTCGACGCGCCGACGGTCGAGGAGCTG  1860
      L  L  G  V  E  L  P  L  R  A  L  F  D  A  P  T  V  E  E  L

               .         .         .         .         .         .
1861  GCCGCCCGGGTGCGGGCCGAACAGGCCGGCGGCCAGGGCGTCCGGGAGGAGGCGGCGCTC  1920
      A  A  R  V  R  A  E  Q  A  G  G  Q  G  V  R  E  E  A  A  L

               .         .         .         .         .         .
1921  GAGCCGGTGGGCCGGAGCGAGCCGCTGCCGCTGTCGTTCGCACAGCAACGCCTGTGGTTC  1980
      E  P  V  G  R  S  E  P  L  P  L  S  F  A  Q  Q  R  L  W  F

               .         .         .         .         .         .
1981  CTGGACCGCTTGATGCCCGACCGCGCCTTCTACACGATGTGCGACGCGTTCCGCGTCCGG  2040
      L  D  R  L  M  P  D  R  A  F  Y  T  M  C  D  A  F  R  V  R

               .         .         .         .         .         .
2041  GGCGGGATCGACCTGGGTGCGCTGCGGCGGGCCCTGCGGATGCTGGTGGGACGGCACGAG  2100
      G  G  I  D  L  G  A  L  R  R  A  L  R  M  L  V  G  R  H  E

               .         .         .         .         .         .
2101  ACGCTGCGGACGGCGTTCGTCGAGCGGGACGGTGTGCCGTACCAGCTCGTCGGTCCGGCC  2160
      T  L  R  T  A  F  V  E  R  D  G  V  P  Y  Q  L  V  G  P  A

               .         .         .         .         .         .
2161  GACGGGCCCGGTGCGCGGCGCGTGGCCGCTCCCACGCGGGTCGACCTGTCGCTGCTGGAG  2220
      D  G  P  G  A  R  R  V  A  A  P  T  R  V  D  L  S  L  L  E

               .         .         .         .         .         .
2221  CCCGCCGAGCGGGAGGAGGCGGTGCGGAACCTGGTGGCGGCGGAGGCGCGGACCCCGTTC  2280
      P  A  E  R  E  E  A  V  R  N  L  V  A  A  E  A  R  T  P  F

               .         .         .         .         .         .
2281  CGGCCGGCGGACGGCGCGCTGCTGCGCGTGGTGGTGGCCCGGCTGGCGGACGATGATCAC  2340
      R  P  A  D  G  A  L  L  R  V  V  V  A  R  L  A  D  D  D  H

               .         .         .         .         .         .
2341  GTGCTGGTGGTCAGCACGCACCACATCGTCTCCGACGCCTGGTCCGTGGGTGTGCTGGTG  2400
      V  L  V  V  S  T  H  H  I  V  S  D  A  W  S  V  G  V  L  V

               .         .         .         .         .         .
2401  GACGAACTCGGACGGCTGTACCGCGAGTGCGTCACCGGAGATCCCGCCGCGCTGCCCCCG  2460
      D  E  L  G  R  L  Y  R  E  C  V  T  G  D  P  A  A  L  P  P

               .         .         .         .         .         .
2461  CCGGCCGTCCAGTACGCCGACTTCGCGGTCTGGCAGCGGGCCTGGATGGCCGGTCCGGTG  2520
      P  A  V  Q  Y  A  D  F  A  V  W  Q  R  A  W  M  A  G  P  V

               .         .         .         .         .         .
2521  CAGGAGGAGCATCTCGCGTACTGGAAGCGGGCCTTGGACGGCGCTCCCTCGGTGCTGCGG  2580
      Q  E  E  H  L  A  Y  W  K  R  A  L  D  G  A  P  S  V  L  R

               .         .         .         .         .         .
2581  CTGCCCATGGACCACCCGCGGCCCGCCGTGCAGTCCGAGCGGGGCGAGACGGTCGGGTTC  2640
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          L   P   M   D   H   P   R   P   A   V   Q   S   E   R   G   E   T   V   G   F

                      .         .         .         .         .         .
2641  GCGCTGCCCGACGCGCTGGTCGCCGCGCTGGAGAAGCTGGGCCGGGAGCAGGGCGCCACC   2700
      A   L   P   D   A   L   V   A   A   L   E   K   L   G   R   E   Q   G   A   T

                      .         .         .         .         .         .
2701  CTGTTCATGACGCTGCTCGGCGCCTTCCAGGTCCTGCTGGCGCGTCACGCCGGGCAAGAG   2760
      L   F   M   T   L   L   G   A   F   Q   V   L   L   A   R   H   A   G   Q   E

                      .         .         .         .         .         .
2761  GACATCGTGGTCGGCGTGCCGGCGGCGGGGCGCACCCGGACCGAGACGGAACCTCTGGTC   2820
      D   I   V   V   G   V   P   A   A   G   R   T   R   T   E   T   E   P   L   V

                      .         .         .         .         .         .
2821  GGCTTCTTCGTCAACACGCTTCCCTTGCGGGCGATCTGCGCTCCGGGCCTGTCGTTCCGG   2880
      G   F   F   V   N   T   L   P   L   R   A   I   C   A   P   G   L   S   F   R

                      .         .         .         .         .         .
2881  GACCTGCTGGACCAGGTGCGCGAGGCCGCCCTCGGCGCCTTCGCCCATCAGGACCTCCCC   2940
      D   L   L   D   Q   V   R   E   A   A   L   G   A   F   A   H   Q   D   L   P

                      .         .         .         .         .         .
2941  TTCGAGGCGCTGGTCGAGGCGCTCGCACCCGAGCGCGACCTCGGCCACAATCCCCTCGTC   3000
      F   E   A   L   V   E   A   L   A   P   E   R   D   L   G   H   N   P   L   V

                      .         .         .         .         .         .
3001  CAGGTCACCTTCCAGCTCCTGGGCACACCGGCGGCGCGGCCGGACCTGATCGGGACGGAG   3060
      Q   V   T   F   Q   L   L   G   T   P   A   A   R   P   D   L   I   G   T   E

                      .         .         .         .         .         .
3061  GTCGAGCGGTACCCGGTCCAGGAGGCCGTCTCGCAGTTCGACCTGTCCCTGGACATCAAG   3120
      V   E   R   Y   P   V   Q   E   A   V   S   Q   F   D   L   S   L   D   I   K

                      .         .         .         .         .         .
3121  CGGGCCGACGACGGTTCCTACCGGGGGATCCTGAACTACTGCCCCGACCTGTTCGACCGA   3180
      R   A   D   D   G   S   Y   R   G   I   L   N   Y   C   P   D   L   F   D   R

                      .         .         .         .         .         .
3181  CGCCGCATGGAGGTGCTGGTCGGCCACTACCTGACGCTGCTCGGCGCCGCCGCCGCGGAC   3240
      R   R   M   E   V   L   V   G   H   Y   L   T   L   L   G   A   A   A   A   D

                      .         .         .         .         .         .
3241  CCGGGCCGCCCGATCGGTGAGCTGCCGCTGTCCGACGGGGCCGAACGGCTGCGGCTGCTC   3300
      P   G   R   P   I   G   E   L   P   L   S   D   G   A   E   R   L   R   L   L

                      .         .         .         .         .         .
3301  GACGGGTTCGGGAAGCGGGACGCGGCGTACGCCGGGCCGGGAAGCGTTCCGGAGCGGTTC   3360
      D   G   F   G   K   R   D   A   A   Y   A   G   P   G   S   V   P   E   R   F

                      .         .         .         .         .         .
3361  GCGGAGGTGGCGCGGACGGCACCGGACGCGCGGGCGGTGACGTGTGGCGCGACAACGCTC   3420
      A   E   V   A   R   T   A   P   D   A   R   A   V   T   C   G   A   T   T   L

                      .         .         .         .         .         .
3421  ACCTTCGCCGAGCTGAACGACCGGGTGGAGCGCCTGGCACAGGCACTGCTCGGCGCCGGG   3480
      T   F   A   E   L   N   D   R   V   E   R   L   A   Q   A   L   L   G   A   G

                      .         .         .         .         .         .
3481  GTCACCCGCGAGACGCCGGTCGCGGTCCGCCTGCCCCGTTCCACCGACAGCGTCGTCGCC   3540
      V   T   R   E   T   P   V   A   V   R   L   P   R   S   T   D   S   V   V   A

                      .         .         .         .         .         .
3541  CTGCTGGCCGTCATGCGGGCGGGCGGCGTCTACGTCCCCCTGGACCCCGACTGGCCCGCG   3600
      L   L   A   V   M   R   A   G   G   V   Y   V   P   L   D   P   D   W   P   A

                      .         .         .         .         .         .
3601  GACCGCACCGCCTACATCCTGGACGACACCGCGGCCTCCGTCGTCATCACCCGCGACCTG   3660
      D   R   T   A   Y   I   L   D   D   T   A   A   S   V   V   I   T   R   D   L

                      .         .         .         .         .         .
3661  CCCGCACTCCCCGGTCGCCTCCACGTCGACCCGCGCCGGCCCGCGGCCGACGGCCTGGTA   3720
      P   A   L   P   G   R   L   H   V   D   P   R   R   P   A   A   D   G   L   V

                      .         .         .         .         .         .
3721  CCCGCGCCCCGCATCGACCCCGATCAGGCCGCGTACGTCATCTACACGTCCGGCTCGACG   3780
      P   A   P   R   I   D   P   D   Q   A   A   Y   V   I   Y   T   S   G   S   T

                      .         .         .         .         .         .
3781  GGCGCGCCGAAGGGCGTCGTCGTCCGGCACCGCTCCCTGAACCACCTCACCAGCGCCCTG   3840
      G   A   P   K   G   V   V   V   R   H   R   S   L   N   H   L   T   S   A   L
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               .         .         .         .         .         .
3841  CAGGCCACCTTTCTCGGCCACGACCCGTATCTCGCCGGGGCCGACGGCGTACCGCCCGGG   3900
      Q  A  T  F  L  G  H  D  P  Y  L  A  G  A  D  G  V  P  P  G

               .         .         .         .         .         .
3901  GACGCGAAGCTGCGTACGACGCTCACCGCGCCCTTCACGTTCGACGCGTCCATGGAGCAA   3960
      D  A  K  L  R  T  T  L  T  A  P  F  T  F  D  A  S  M  E  Q

               .         .         .         .         .         .
3961  CTGAGCTGGATGCTGGCCGGTCACGAGCTGTTCATCGTGCCCGAGGACGTGCGGCGCGAC   4020
      L  S  W  M  L  A  G  H  E  L  F  I  V  P  E  D  V  R  R  D

               .         .         .         .         .         .
4021  CCCTCGGCGCTGGTCCGGTTCGTCCGGGAGCACCGGATCGACGTCATCGACACGACCTCC   4080
      P  S  A  L  V  R  F  V  R  E  H  R  I  D  V  I  D  T  T  S

               .         .         .         .         .         .
4081  TCGCAGCTCGAACTCCTCGTATCGCACGGGCTGTTGGACGGAGAGTGGGCGCCGTCCATG   4140
      S  Q  L  E  L  L  V  S  H  G  L  L  D  G  E  W  A  P  S  M

               .         .         .         .         .         .
4141  GTCATGGTGGGTGGCGAGGCGGTCTCGCCGTCGCTGTGGCGGACCTTGCGGGACCAGCGG   4200
      V  M  V  G  G  E  A  V  S  P  S  L  W  R  T  L  R  D  Q  R

               .         .         .         .         .         .
4201  CGCACTCGCTGTTTCAACCTGTACGGGCCTACGGAGGCGACGGTCGACGCCACCTGCCAC   4260
      R  T  R  C  F  N  L  Y  G  P  T  E  A  T  V  D  A  T  C  H

               .         .         .         .         .         .
4261  GACCTGTCCGACCCCGCCGACGTCCCCGTCATCGGCACCCCACTCCCCCACACCCACGTC   4320
      D  L  S  D  P  A  D  V  P  V  I  G  T  P  L  P  H  T  H  V

               .         .         .         .         .         .
4321  CGCGTGCTCGACGACCGACTGCGACCCGTACCCGTGGGCGTCGCCGGCGAGATCTACCTC   4380
      R  V  L  D  D  R  L  R  P  V  P  V  G  V  A  G  E  I  Y  L

               .         .         .         .         .         .
4381  GGCGGAACCGGCCTGGCCCGCGGCTACCTCAACCGCCCCGCCCTCACCGCCCGACGCTTC   4440
      G  G  T  G  L  A  R  G  Y  L  N  R  P  A  L  T  A  R  R  F

               .         .         .         .         .         .
4441  GTCGCCGACCCCTACCCCGACACCCCCGGCAGCCGCCTGTACCGCACCGGCGACCGCGCC   4500
      V  A  D  P  Y  P  D  T  P  G  S  R  L  Y  R  T  G  D  R  A

               .         .         .         .         .         .
4501  CGCTGGCGCCCCGACGGCACCCTCGAATACCTGGGACGCACCGACGACCAAATCAAGATC   4560
      R  W  R  P  D  G  T  L  E  Y  L  G  R  T  D  D  Q  I  K  I

               .         .         .         .         .         .
4561  CGCGGCTTCCGCGTCGAACCCGGCGAAATCGAGGCCGTCCTCACCCACCACCCCGCCGTC   4620
      R  G  F  R  V  E  P  G  E  I  E  A  V  L  T  H  H  P  A  V

               .         .         .         .         .         .
4621  AAGGAAGCCGCCGTCGTCGACGACGCGCACGCGCGGCTGGTCGCCTACGTCACGCTCGCG   4680
      K  E  A  A  V  V  D  D  A  H  A  R  L  V  A  Y  V  T  L  A

               .         .         .         .         .         .
4681  GAAGGCGGCGGCGCCGGCCCCACCGACGTACGCCGGTTCGCGCAGGGGCGGCTGCCCGCC   4740
      E  G  G  G  A  G  P  T  D  V  R  R  F  A  Q  G  R  L  P  A

               .         .         .         .         .         .
4741  CACATGGTGCCGTCGGCGGTGGTCGTCCTGGAGGCGCTGCCACTGACGTCGAACGGAAAG   4800
      H  M  V  P  S  A  V  V  V  L  E  A  L  P  L  T  S  N  G  K

               .         .         .         .         .         .
4801  CTGGACCGCGCGCGCCTGCCGGCGCCCGCGGCGGGCAGACCGGAACTGGATGTCCGCTTC   4860
      L  D  R  A  R  L  P  A  P  A  A  G  R  P  E  L  D  V  R  F

               .         .         .         .         .         .
4861  GTGGCGCCGCGCGACATGGTGGAGGAGGTCGTGGCGCAGGTCTGGTGCGCCGTGCTGGGC   4920
      V  A  P  R  D  M  V  E  E  V  V  A  Q  V  W  C  A  V  L  G

               .         .         .         .         .         .
4921  GTCGACCGGGTCGGTGTGCACGACGACTTCTTCGAGCTGGGCGGGCACTCGTTGCTGGTG   4980
      V  D  R  V  G  V  H  D  D  F  F  E  L  G  G  H  S  L  L  V

               .         .         .         .         .         .
4981  GTCCAGGTGATGACCCGGATACGAAAGCTGCTCGGCGTCGAGGTGCCGTTGCGGGAGCTG   5040
      V  Q  V  M  T  R  I  R  K  L  L  G  V  E  V  P  L  R  E  L

               .         .         .         .         .         .
5041  TTCGACGCCGCGACGGTCGAGGAGCTCGCCGCCCGCGTCCGCGCCGCACGGACCGAGGGC   5100
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      F  D  A  A  T  V  E  E  L  A  A  R  V  R  A  A  R  T  E  G

               .         .         .         .         .         .
5101  CTCGGCCGGGGGGCCGCCCCGCCCCTCGGGCCGGTGGACCGGAGCGGGCCGCTGCCGCTG  5160
      L  G  R  G  A  A  P  P  L  G  P  V  D  R  S  G  P  L  P  L

               .         .         .         .         .         .
5161  TCGTTCGCGCAGCAACGCCTTTGGTACCTCGATCAGTTGGCGCCCGACAGTGTCTCCTAC  5220
      S  F  A  Q  Q  R  L  W  Y  L  D  Q  L  A  P  D  S  V  S  Y

               .         .         .         .         .         .
5221  AACATGTGCGACGCCTACCGGGTCCGCGGCCCTCTCGACCTGGACGCGCTGCGGCGGGCG  5280
      N  M  C  D  A  Y  R  V  R  G  P  L  D  L  D  A  L  R  R  A

               .         .         .         .         .         .
5281  CTGCGGACGCTGGTCGAGCGGCACGAGACGCTGCGGACGGCGTTCGTCGAGCGGGACGGG  5340
      L  R  T  L  V  E  R  H  E  T  L  R  T  A  F  V  E  R  D  G

               .         .         .         .         .         .
5341  GTGCCCCACCAGGTGGTCTCGGCGCCCGACGCGCCGGCCGCGCGGCGCGCGGCGGAGGTC  5400
      V  P  H  Q  V  V  S  A  P  D  A  P  A  A  R  R  A  A  E  V

               .         .         .         .         .         .
5401  GTGCGGATCGAGGCGGCCGGGCGGACCGACGAGGCGGTGCGGGACCTGGTGGCCGCGGAG  5460
      V  R  I  E  A  A  G  R  T  D  E  A  V  R  D  L  V  A  A  E

               .         .         .         .         .         .
5461  GCGCGCACCCCGTTCCGGCCGGCGGACGGCGCGCTGATGCGCGTGGCGGTGGCCCGGCTG  5520
      A  R  T  P  F  R  P  A  D  G  A  L  M  R  V  A  V  A  R  L

               .         .         .         .         .         .
5521  GCGGACGACGATCACGTGCTGGTGGTCACCACGCACCACATCGTCTCCGACGGCTGGTCG  5580
      A  D  D  D  H  V  L  V  V  T  T  H  H  I  V  S  D  G  W  S

               .         .         .         .         .         .
5581  GTCGACATCCTGGTGGACGAATTGGGGCGCCTGTACCGGGAACACGTCACGGGTGACCCC  5640
      V  D  I  L  V  D  E  L  G  R  L  Y  R  E  H  V  T  G  D  P

               .         .         .         .         .         .
5641  GCCGGGCTCCCTCCGCTCGACGTCCAGTACGCCGACTTCGCCGTCTGGCAGCGGTCCTGG  5700
      A  G  L  P  P  L  D  V  Q  Y  A  D  F  A  V  W  Q  R  S  W

               .         .         .         .         .         .
5701  ATGACCGGCCCCGTGCGGGAGGAGCACCTCGCGTACTGGAAGCGGGCCCTGGACGGGGCA  5760
      M  T  G  P  V  R  E  E  H  L  A  Y  W  K  R  A  L  D  G  A

               .         .         .         .         .         .
5761  CCCTCGGTCCTGCGGCTGCCGGCGGACCATCCGCGTCCCGCCGTCCAGTCCCAGCGGGGC  5820
      P  S  V  L  R  L  P  A  D  H  P  R  P  A  V  Q  S  Q  R  G

               .         .         .         .         .         .
5821  GAGACCGTCGAGTTCCCCCTGCCCGCACCACTGGTCGCGCGGCTGGAAGCGCTCTGCCGG  5880
      E  T  V  E  F  P  L  P  A  P  L  V  A  R  L  E  A  L  C  R

               .         .         .         .         .         .
5881  GAGCAGGGCGTCACCCTGTTCATGGCGCTCTTCGGCGCGTTCCAGGTGTTGCTGGCGCGC  5940
      E  Q  G  V  T  L  F  M  A  L  F  G  A  F  Q  V  L  L  A  R

               .         .         .         .         .         .
5941  TACAGCGGTCAGGACGACGTGGTCGTGGGCGTGCCGACGGCGAACCGCACCCGCGCGGAG  6000
      Y  S  G  Q  D  D  V  V  V  G  V  P  T  A  N  R  T  R  A  E

               .         .         .         .         .         .
6001  ACCGAGCCCCTGGTCGGCTTCTTCGTCAACACCCTTCCGGTACGGGTCGCGTGCTCGCCG  6060
      T  E  P  L  V  G  F  F  V  N  T  L  P  V  R  V  A  C  S  P

               .         .         .         .         .         .
6061  GAGCTGTCGTTCCGCGCCCTGCTCGACCGGGTCCGCGAGGCCGCGCTGGGCGCCTTCGCC  6120
      E  L  S  F  R  A  L  L  D  R  V  R  E  A  A  L  G  A  F  A

               .         .         .         .         .         .
6121  CATCAGGACCTGCCCTTCGAGGCGCTGGTCGAGGCGCTCGCGCCCGAGCGCGACCTGGGC  6180
      H  Q  D  L  P  F  E  A  L  V  E  A  L  A  P  E  R  D  L  G

               .         .         .         .         .         .
6181  CACCACCCTCTCGTGCAGGTCACCTTCCAACTCCTCGACGCTCCCGACGAGAGGCTCGTC  6240
      H  H  P  L  V  Q  V  T  F  Q  L  L  D  A  P  D  E  R  L  V

               .         .         .         .         .         .
6241  CTGCACGGCACGGACTGCGTCTCGCTCGGCTTCGGCGGTGTGACCAGCCGGTTCGACCTG  6300
      L  H  G  T  D  C  V  S  L  G  F  G  G  V  T  S  R  F  D  L
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               .         .         .         .         .         .
6301  TCCCTCGACGTCGTCTCGGGGCGGCGGGGGAAGCGGTGCGTGCTGACGTACTGTCCCGAC  6360
      S  L  D  V  V  S  G  R  R  G  K  R  C  V  L  T  Y  C  P  D

               .         .         .         .         .         .
6361  CTGTTCGACCGGCCCCGCATGGAGGTGCTGGCCGGCCACTACCTGACCCTGCTCGGCGCG  6420
      L  F  D  R  P  R  M  E  V  L  A  G  H  Y  L  T  L  L  G  A

               .         .         .         .         .         .
6421  GCGGCCGACGATCCCGGTCTCCGCGTCGGCGACCTCCCGCTGAGCGACGACGTCGAACGC  6480
      A  A  D  D  P  G  L  R  V  G  D  L  P  L  S  D  D  V  E  R

               .         .         .         .         .         .
6481  CTGCGCCTGCTGGGCGGGTCCCGCCCGCGGTACCTGCCCGCGCCCGGGGCGGAGACCGTC  6540
      L  R  L  L  G  G  S  R  P  R  Y  L  P  A  P  G  A  E  T  V

               .         .         .         .         .         .
6541  CCTGACGCCTTCGCCGCGCAGGTGCGGGCGACACCGGACGCGCCCGCGCTGGTCCACGGG  6600
      P  D  A  F  A  A  Q  V  R  A  T  P  D  A  P  A  L  V  H  G

               .         .         .         .         .         .
6601  GACTCGACGCTGACGTTCGCCGAGCTGGACACCCGGGTCACCGCCCTGGCCGTGCGGTTG  6660
      D  S  T  L  T  F  A  E  L  D  T  R  V  T  A  L  A  V  R  L

               .         .         .         .         .         .
6661  CGGCGCTGCGGCGTGGCCGCCGAGACGCCGGTCGCGGTGTGCCTGCCGCGCTCCGCCGAC  6720
      R  R  C  G  V  A  A  E  T  P  V  A  V  C  L  P  R  S  A  D

               .         .         .         .         .         .
6721  GCCGTCGTGGCCCTCCTGGCCGTCCTGCGGGCGGGCGGCGTCTATGTGCCAGTGGATCCG  6780
      A  V  V  A  L  L  A  V  L  R  A  G  G  V  Y  V  P  V  D  P

               .         .         .         .         .         .
6781  GAGTGGCCCTCCGGCCGCGTCGCCCACGTCCTCGACGAGACCGCGGCCCCCGTCGTCATC  6840
      E  W  P  S  G  R  V  A  H  V  L  D  E  T  A  A  P  V  V  I

               .         .         .         .         .         .
6841  ACCCGCGACCTGCCCGCCGATCCCGGCCGCGTCCACCTCGACCCGCGCCAGGCCCCGGCC  6900
      T  R  D  L  P  A  D  P  G  R  V  H  L  D  P  R  Q  A  P  A

               .         .         .         .         .         .
6901  GACGACCGGGATCCCCTGCCGCGCCTCCACCGCGACCAGGCCGCGTACATCATCTTCACC  6960
      D  D  R  D  P  L  P  R  L  H  R  D  Q  A  A  Y  I  I  F  T

               .         .         .         .         .         .
6961  TCGGGCTCCACCGGCGCCCCCAAGGGCGTCGTCGTCCGACACGGCTCCCTGTACCACCTC  7020
      S  G  S  T  G  A  P  K  G  V  V  V  R  H  G  S  L  Y  H  L

               .         .         .         .         .         .
7021  CTGGGCCACGTACGGCGCATGGCGGAGGGCGGCCCCCGGCGGAACGTCGCGCACACCACC  7080
      L  G  H  V  R  R  M  A  E  G  G  P  R  R  N  V  A  H  T  T

               .         .         .         .         .         .
7081  GCGATGACCTTCGACCCGTCGCTGGAACAGTTCCTGTGGCTCGTCGCCGGACACACCCTG  7140
      A  M  T  F  D  P  S  L  E  Q  F  L  W  L  V  A  G  H  T  L

               .         .         .         .         .         .
7141  CACGTCGCGCCCGAGGAGGTGCGCCGCGATCCCGAGGCGCTGGTGGCCCTGGTGCGGCGC  7200
      H  V  A  P  E  E  V  R  R  D  P  E  A  L  V  A  L  V  R  R

               .         .         .         .         .         .
7201  GCCGCGATCGACGTCCTCAACGTCACCCCGTCCCACCTGACCCTGCTGATCGAGGCCGGG  7260
      A  A  I  D  V  L  N  V  T  P  S  H  L  T  L  L  I  E  A  G

               .         .         .         .         .         .
7261  CTGCTGGAGGGCGACCGGGTGCCGGGTACGGTCCTGGTGGGTGGCGAGGCGGTGCCCGCG  7320
      L  L  E  G  D  R  V  P  G  T  V  L  V  G  G  E  A  V  P  A

               .         .         .         .         .         .
7321  GCGCTGTGGCGGACCCTGCGCGAACGGACGGGAGCCACCCGCTTCTTCAACCTGTACGGG  7380
      A  L  W  R  T  L  R  E  R  T  G  A  T  R  F  F  N  L  Y  G

               .         .         .         .         .         .
7381  CCTACGGAGGCGACGGTCGACGCCACCTGCCACGACCTGTCCGACCCCGCCGACGTCCCC  7440
      P  T  E  A  T  V  D  A  T  C  H  D  L  S  D  P  A  D  V  P

               .         .         .         .         .         .
7441  GTCATCGGCACCCCACTCCCCCACACCCACGTCCGCGTGCTCGACGACCGACTGCGACCC  7500
      V  I  G  T  P  L  P  H  T  H  V  R  V  L  D  D  R  L  R  P

               .         .         .         .         .         .
7501  GTACCCGTGGGCGTCGCCGGCGAAATCTACCTCGGCGGAACCGGCCTGGCCCGCGGCTAC  7560
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       V  P  V  G  V  A  G  E  I  Y  L  G  G  T  G  L  A  R  G  Y

                .         .         .         .         .         .
7561   CTCAACCGCCCCGCCCTCACCGCCCAACGCTTCGTCGCCGACCCCTACCCCGACACCCCC    7620
       L  N  R  P  A  L  T  A  Q  R  F  V  A  D  P  Y  P  D  T  P

                .         .         .         .         .         .
7621   GGCAGCCGCCTGTACCGCACCGGCGACCGCGCCCGCTGGCGCCCCGACGGCACCCTCGAA    7680
       G  S  R  L  Y  R  T  G  D  R  A  R  W  R  P  D  G  T  L  E

                .         .         .         .         .         .
7681   TACCTGGGACGCACCGACGACCAAATCAAGATCCGCGGCTTCCGCGTCGAACCCGGCGAG    7740
       Y  L  G  R  T  D  D  Q  I  K  I  R  G  F  R  V  E  P  G  E

                .         .         .         .         .         .
7741   ATCGAAGCCGTCCTCACCCACCACCCCGCCGTCAAGGAAGCCGCCGTCACCGTGGCCACC    7800
       I  E  A  V  L  T  H  H  P  A  V  K  E  A  A  V  T  V  A  T

                .         .         .         .         .         .
7801   GACGACGGTGCCGCCCGGCTGGTCGCCCTCGTCGTCCCCGCCCCCCGCGCCCCGCACGGC    7860
       D  D  G  A  A  R  L  V  A  L  V  V  P  A  P  R  A  P  H  G

                .         .         .         .         .         .
7861   GATTCGGCCGACGGCGCCCCGGACGCCCAGGTCGAGGAGTGGAACGCCGTCTTCGAGGCG    7920
       D  S  A  D  G  A  P  D  A  Q  V  E  E  W  N  A  V  F  E  A

                .         .         .         .         .         .
7921   ACCCACACCGACGCCGCCGACGGCGAACTCACCTTCAACATCAAGGGCTGGAACGACAGC    7980
       T  H  T  D  A  A  D  G  E  L  T  F  N  I  K  G  W  N  D  S

                .         .         .         .         .         .
7981   CTCACCGGTGCGCCGATCCCCGCCGAACACATGCGGGAATGGGTCGACACCACCGTCGCC    8040
       L  T  G  A  P  I  P  A  E  H  M  R  E  W  V  D  T  T  V  A

                .         .         .         .         .         .
8041   CGGCTCCTGGAACGGCCGGCCGAGCGCGTCCTGGAGATCGGCAGTGGCACCGGGCTGCTG    8100
       R  L  L  E  R  P  A  E  R  V  L  E  I  G  S  G  T  G  L  L

                .         .         .         .         .         .
8101   ATGTGGCGGCTGCTGCCGCACGTCACCGAGTACACCGGAACCGACTTCTCGCGGCCCGCC    8160
       M  W  R  L  L  P  H  V  T  E  Y  T  G  T  D  F  S  R  P  A

                .         .         .         .         .         .
8161   GTGGACTGGCTCCGGGACGGGCTGCGCCGCCGCCCCGCGCACCGGGTACGGCTGCTGCAC    8220
       V  D  W  L  R  D  G  L  R  R  R  P  A  H  R  V  R  L  L  H

                .         .         .         .         .         .
8221   CGCGAGGCGACCGACTTCACCGGCGTCCGCGCCGCGTCCACCGACCTCGTCGTCGTCAAC    8280
       R  E  A  T  D  F  T  G  V  R  A  A  S  T  D  L  V  V  V  N

                .         .         .         .         .         .
8281   TCGGTCGTCCAGTACTTCCCCGACCGCGCCTACCTCGACACCGTCCTGGCCCGCGCCCTC    8340
       S  V  V  Q  Y  F  P  D  R  A  Y  L  D  T  V  L  A  R  A  L

                .         .         .         .         .         .
8341   GACGCCACGGCCGACCGAGGGCGCGTCTTCGTGGGCGACGTGCGCAACCTGGCCCTCGCC    8400
       D  A  T  A  D  R  G  R  V  F  V  G  D  V  R  N  L  A  L  A

                .         .         .         .         .         .
8401   CCGCAGTTCTACGCCCGTCAGGCCCTCGCCCACGCCGGTCCGGGCGCGGCGGCGCGGGAC    8460
       P  Q  F  Y  A  R  Q  A  L  A  H  A  G  P  G  A  A  A  R  D

                .         .         .         .         .         .
8461   GTGGCGCGCGCCGCCGGCGAGTTCGCGGCCATGGACGGCGAGTTGCTGGTGTCCCCCGCG    8520
       V  A  R  A  A  G  E  F  A  A  M  D  G  E  L  L  V  S  P  A

                .         .         .         .         .         .
8521   TACTTCGCCGCGCTCGCCGCCCGCTCGCCCCGCGTCACCGGCGTCGAGATCCTGCCCCGC    8580
       Y  F  A  A  L  A  A  R  S  P  R  V  T  G  V  E  I  L  P  R

                .         .         .         .         .         .
8581   CGGGGACGGCACCGCAACGAGATGAGCCTGTACCGCTACGACGTGGTGCTGCACGTGGGC    8640
       R  G  R  H  R  N  E  M  S  L  Y  R  Y  D  V  V  L  H  V  G

                .         .         .         .         .         .
8641   GGTGACCGCCCGGCGGCCCCGGAGGCGGAGGTGCTCACCTGGGGCGACCAGGTGCACGAC    8700
       G  D  R  P  A  A  P  E  A  E  V  L  T  W  G  D  Q  V  H  D

                .         .         .         .         .         .
8701   CTCGCGTCGCTGTCCGCCCGCCTCGGCCGCGGGGGCCCGGACGCCCTGCTCGTGCGCGGC    8760
       L  A  S  L  S  A  R  L  G  R  G  G  P  D  A  L  L  V  R  G
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               .         .         .         .         .         .
8761  GTCGCCAACGACCGTCTGACGCGGGACAACGAGCTGCTCGACGCACCCGCCCGCACGACG  8820
      V  A  N  D  R  L  T  R  D  N  E  L  L  D  A  P  A  R  T  T

               .         .         .         .         .         .
8821  GCCGTCGAGCCCGAGGACCTGTGGGGGCTGGCGGACTCCACCCCCTACCGGGTGAGCGTC  8880
      A  V  E  P  E  D  L  W  G  L  A  D  S  T  P  Y  R  V  S  V

               .         .         .         .         .         .
8881  AGCTGGGCCGCCGCCGATCCGCGGGGCGCGATGGACGTCCTGCTGGTCCGGCGGGACGCC  8940
      S  W  A  A  A  D  P  R  G  A  M  D  V  L  L  V  R  R  D  A

               .         .         .         .         .         .
8941  CACGACGACGGTCCGCTGCTCGTCCCCCACCCCGTACCGGAGCCCTCGGCACCGCTGACG  9000
      H  D  D  G  P  L  L  V  P  H  P  V  P  E  P  S  A  P  L  T

               .         .         .         .         .         .
9001  AACACGCCGACCCGGCACCCGTCCGCGCGGCAAGGGGGCTCGGCCGCGGACGGGCTGCGT  9060
      N  T  P  T  R  H  P  S  A  R  Q  G  G  S  A  A  D  G  L  R

               .         .         .         .         .         .
9061  TCCTGGCTCGCCGAGCGGCTTCCCGCGCACCTGCTGCCCGCGAGGATCACCGAGGTGGAC  9120
      S  W  L  A  E  R  L  P  A  H  L  L  P  A  R  I  T  E  V  D

               .         .         .         .         .         .
9121  GCGCTGCCCCGCACCGGCACCGGCAAGCTCGACCGGGGCGCGCTCGGCGGACTCGTGACC  9180
      A  L  P  R  T  G  T  G  K  L  D  R  G  A  L  G  G  L  V  T

               .         .         .         .         .         .
9181  GCGGGCCGTGGCGCCCGGGCGGGCGACCGCCCCGCCACCGCCCCCCGTACGGGTCTCGAA  9240
      A  G  R  G  A  R  A  G  D  R  P  A  T  A  P  R  T  G  L  E

               .         .         .         .         .         .
9241  CGGACCCTGGCCGACGCGTGGGCGCGGGTGCTCGGCCTCCCCGAAGTCGGCGTGCACGAG  9300
      R  T  L  A  D  A  W  A  R  V  L  G  L  P  E  V  G  V  H  E

               .         .         .         .         .         .
9301  AACTTCTTCGCCCTCGGCGGCGACTCCCTCCTCGCCGTCAGGGCTGTCGCCCGGTGCCGC  9360
      N  F  F  A  L  G  G  D  S  L  L  A  V  R  A  V  A  R  C  R

               .         .         .         .         .         .
9361  CGTGCCGGGGTCCGACTGACCGTCCGGCAGTTGCTGAGCGAGCAGACCGTCGCCGCGCTC  9420
      R  A  G  V  R  L  T  V  R  Q  L  L  S  E  Q  T  V  A  A  L

               .         .         .         .         .         .
9421  GCGGCGGCCCTCGAGGAGGAGTCTCAATGATGAAGTCAAGCCGCTTGCGCGACCGGCAGC  9480
      A  A  A  L  E  E  E  S  Q  *
                                M  M  K  S  S  R  L  R  D  R  Q  L
                                (orf33)
               .         .         .         .         .         .
9481  TCGGGGGTGAAGACCCGGTTGTCGCGCAGGAGAGCCCACAGGACGCTGGCCCGACGCCGT  9540
       G  G  E  D  P  V  V  A  Q  E  S  P  Q  D  A  G  P  T  P  C

               .         .         .         .         .         .
9541  GCCAGGGCGATGACGGCTTGAACGTGTTTGCAGCCCTCGCCGCGCTTCTTGAGGTAGAAG  9600
       Q  G  D  D  G  L  N  V  F  A  A  L  A  A  L  L  E  V  E  V

               .         .         .         .         .         .
9601  TCCCGGTTCGGCCCCTCCCGCATCATGCTGGTTTGGGCCGACATGTAGAACACTCGTCGC  9660
       P  V  R  P  L  P  H  H  A  G  L  G  R  H  V  E  H  S  S  Q

               .         .         .         .         .         .
9661  AGGCGGCGGCTGTAGCGCTTGGGCCGATGCAGGTTGCCAGTGCGACGACCGGAGTCGCGG  9720
       A  A  A  V  A  L  G  P  M  Q  V  A  S  A  T  T  G  V  A  G

               .         .         .         .         .         .
9721  GGGACGGGCACCAGGCCGGCCGCCGAGGCCAGGTGACCGGCGTCGGCGTAGGCCGTGAGG  9780
       D  G  H  Q  A  G  R  R  G  Q  V  T  G  V  G  V  G  R  E  V

               .         .         .         .         .         .
9781  TCGCCGGCGGCGACGACGAACTCGGCGCCGAGGATCGGCCCCATGCCCGGCAGAGACTCG  9840
       A  G  G  D  D  E  L  G  A  E  D  R  P  H  A  R  Q  R  L  D

               .         .         .         .         .         .
9841  ATGATCTCGGCCTGTGGATGGCTGCGGAACGTCTCGCGGATCTGCTGGTCAATCCGCTTC  9900
       D  L  G  L  W  M  A  A  E  R  L  A  D  L  L  V  N  P  L  Q

               .         .         .         .         .         .
9901  AGACGGTCGTCCAGGGCCAGGATCTGCGCGGCCAGGTCAGCCACGATCTGGGCGGCGACG  9960
       T  V  V  Q  G  Q  D  L  R  G  Q  V  S  H  D  L  G  G  D  V

               .         .         .         .         .         .
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 9961  TCCTCCCCGGGCAGCGCGGTCTGCTGAGCCTGGGCAGCCTCCAGCGCCGTCGCGGCGACG   10020
         L  P  G  Q  R  G  L  L  S  L  G  S  L  Q  R  R  R  G  D  G

                .         .         .         .         .         .
10021  GCGTCGGCACCGCGCACGCCTCGGTTGGCCAGCCAGGCCGTCAGCCGGGCCCGGCCGCGG   10080
         V  G  T  A  H  A  S  V  G  Q  P  G  R  Q  P  G  P  A  A  A

                .         .         .         .         .         .
10081  CGGCGGAGAGCTGCCGGGGTCTGGTAGCCCGTCAGCAGGACCAGCGCGCCCTTCTGCGAG   10140
         A  E  S  C  R  G  L  V  A  R  Q  Q  D  Q  R  A  L  L  R  A

                .         .         .         .         .         .
10141  CTGTAGTCGAAGGCCCGTTCCAGCGCGGGGAAGACGCCGGTCAGCGTGTCGCGGAGACGG   10200
         V  V  E  G  P  F  Q  R  G  E  D  A  G  Q  R  V  A  E  T  V

                .         .         .         .         .         .
10201  TTGATCATCCTGACCCGGTCGGCCACGAGGTCGGAACGGTGGGCGGTCAGCAGCGCGAGG   10260
         D  H  P  D  P  V  G  H  E  V  G  T  V  G  G  Q  Q  R  E  V

                .         .         .         .         .         .
10261  TCGGCGGCCAGCTGGGCGGGCACGTCGATCGACGCGAAGTCCCGTCGGTTGCGGGCGGTT   10320
         G  G  Q  L  G  G  H  V  D  R  R  E  V  P  S  V  A  G  G  F

                .         .         .         .         .         .
10321  TCGGCGATGACGTAGGCGTCGCGGGCGTCGGTCTTCGCCTCGCCCCGGTAAGCGCCGGAC   10380
         G  D  D  V  G  V  A  G  V  G  L  R  L  A  P  V  S  A  G  H

                .         .         .         .         .         .
10381  ATGCGGTTGACCGTGCGGCCGGGCACGTAGACGGCCTGCTGGCCGTGGGCCGCGAGCAGG   10440
         A  V  D  R  A  A  G  H  V  D  G  L  L  A  V  G  R  E  Q  G

                .         .         .         .         .         .
10441  GCCAGCAGCAGCGCGGAGGACGTGCCGGAGATGTCCACTGCCCAGTGGACCTCGTCGGCC   10500
         Q  Q  Q  R  G  G  R  A  G  D  V  H  C  P  V  D  L  V  G  Q

                .         .         .         .         .         .
10501  AGGTCGAGGATCTCACCCATGGCGGTCAGGATCGCCGACTCATCGTTGCCGATCTTCTTC   10560
         V  E  D  L  T  H  G  G  Q  D  R  R  L  I  V  A  D  L  L  R

                .         .         .         .         .         .
10561  GACCACAGCGTCACACCGGTCTCGTCGACCACCGCCGCCCAGTGATGCCCCTTGCCCGCG   10620
         P  Q  R  H  T  G  L  V  D  H  R  R  P  V  M  P  L  A  R  V

                .         .         .         .         .         .
10621  TCGATCCCGGCCCAGACCCGGGCCCGTCGCTCGCCCACTCGCCCCTCCTCACTCCGAACA   10680
         D  P  G  P  D  P  G  P  S  L  A  H  S  P  L  L  T  P  N  S

                .         .         .         .         .         .
10681  GCATCCCGTCGACCCGAGGAACACCCCGCTGTCATCTCCGTAAAAAGCGACCGAAGCGCA   10740
         I  P  S  T  R  G  T  P  R  C  H  L  R  K  K  R  P  K  R  T

                .         .         .         .         .         .
10741  CATCTCAATCAGCAGCCAGGGCGCCCCGGAGAACCGGGCGGCCACTCCTTGTAAGCCACT   10800
         S  Q  S  A  A  R  A  P  R  R  T  G  R  P  L  L  V  S  H  *

                .         .         .         .         .         .
10801  GACGGCAGAGAACCATAAGCCACACCCGGCCCTCCCGGGCCGCCTAACAACTTACGGAGA   10860

                .         .         .         .         .         .
10861  ACCATGACTGACCTGCCGTTGCGTACCGTCGCACTCACCGGTGAGGAGAGCGCGGAGGTC   10920
          M  T  D  L  P  L  R  T  V  A  L  T  G  E  E  S  A  E  V
          (orf34)
                .         .         .         .         .         .
10921  GACGACCTGCTGCGCACGCTGGCCGACGTGCCGGTCGACTCCACCGTGGGACTGCTGCAC   10980
       D  D  L  L  R  T  L  A  D  V  P  V  D  S  T  V  G  L  L  H

                .         .         .         .         .         .
10981  CGCACCCGGCTCGCCGCACAGGAACTGCCGCTGCGCATCCGCGCCGAGCTCACGGGGATG   11040
       R  T  R  L  A  A  Q  E  L  P  L  R  I  R  A  E  L  T  G  M

                .         .         .         .         .         .
11041  CGGCTCTACGACAGCCCGCGCGCCCTCGTCGTCACGGGCTTCGGCGTCGACGACGAACGG   11100
       R  L  Y  D  S  P  R  A  L  V  V  T  G  F  G  V  D  D  E  R

                .         .         .         .         .         .
11101  ATCGGACCGACCCCCGCGGCCCGTCCCGCCCCGGATCCCGAGCGGACCCGCGACCTGGAG   11160
       I  G  P  T  P  A  A  R  P  A  P  D  P  E  R  T  R  D  L  E

                .         .         .         .         .         .
11161  CTGCTGCTTTTGCTGCACGCGGCCCTGCTCGGCGAGGCGTTCGGCTGGGCGACCCAGCAG   11220
       L  L  L  L  L  H  A  A  L  L  G  E  A  F  G  W  A  T  Q  Q
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                .         .         .         .         .         .
11221  AACGGCCGGCTCGTCCACGACGTGCTGCCCGTTCCCGGTGAGGAGACCGCGCAGATGGGT  11280
       N  G  R  L  V  H  D  V  L  P  V  P  G  E  E  T  A  Q  M  G

                .         .         .         .         .         .
11281  TCCAGCAGCGAGACCGAGCTGCTGTGGCACACCGAGGACGCGTTCCACCCGCTGCGCTGC  11340
       S  S  S  E  T  E  L  L  W  H  T  E  D  A  F  H  P  L  R  C

                .         .         .         .         .         .
11341  GACTACGTGGGCCTGCTGTGCCTGCGCAACCACCAGCGCGCCGCGACCACCGTGGGCTGG  11400
       D  Y  V  G  L  L  C  L  R  N  H  Q  R  A  A  T  T  V  G  W

                .         .         .         .         .         .
11401  CCCGACCTGTCCCGGCTCACCACCGAGGACCGTGCCGTGCTCCTCGAACCCCGCTATCTG  11460
       P  D  L  S  R  L  T  T  E  D  R  A  V  L  L  E  P  R  Y  L

                .         .         .         .         .         .
11461  ATCCGCCCGGACACCTCGCACACGCCCGCGCAGAACGCGACGGGCACGCGGTCCGCCGAG  11520
       I  R  P  D  T  S  H  T  P  A  Q  N  A  T  G  T  R  S  A  E

                .         .         .         .         .         .
11521  CGTTTCGCGGCGATCGCCGAGATGGACGACGCCCCGGAGCGCGTCGCCGTCCTGTTCGGC  11580
       R  F  A  A  I  A  E  M  D  D  A  P  E  R  V  A  V  L  F  G

                .         .         .         .         .         .
11581  GACCCCGAGGACCCGTACCTGCGGATCGACCCGGCCTACATGAGCCCGGCCCCCGGGGAC  11640
       D  P  E  D  P  Y  L  R  I  D  P  A  Y  M  S  P  A  P  G  D

                .         .         .         .         .         .
11641  GCGGCCGCCCGGCGGGCGTACGACACCGTCACCGCGCTCATCGAGGACGAGCTGCGGCAC  11700
       A  A  A  R  R  A  Y  D  T  V  T  A  L  I  E  D  E  L  R  H

                .         .         .         .         .         .
11701  GTCGTCCTGGACGCCGGTTCACTGCTGCTGGTCGACAACTACCAGGCGGTGCACGGCCGC  11760
       V  V  L  D  A  G  S  L  L  L  V  D  N  Y  Q  A  V  H  G  R

                .         .         .         .         .         .
11761  AAGCCGTTCGCCGCCGCCTACGACGGCCGCGACCGCTGGCTCAAACGCGTCAACATCACC  11820
       K  P  F  A  A  A  Y  D  G  R  D  R  W  L  K  R  V  N  I  T

                .         .         .         .         .         .
11821  CGCGACCTGCGCCGTTCCCGGTCCGCGCGGCGGTCGGCCACCTCGCTGCTGGTGTGAGGG  11880
       R  D  L  R  R  S  R  S  A  R  R  S  A  T  S  L  L  V  *

                .         .         .         .         .         .
11881  AGGCACCATGGATTTCCCCCTCACCCGCGTCAACCCCTGGTTCAGCGGCGGCTGCGACGG  11940
              M  D  F  P  L  T  R  V  N  P  W  F  S  G  G  C  D  G
              (orf35)
                .         .         .         .         .         .
11941  CCGCCCCCGGGTGCGGCTGTGCGCGCTGCCGTACGCGGGCGGCACCGCCGCCGTCTTCAA  12000
        R  P  R  V  R  L  C  A  L  P  Y  A  G  G  T  A  A  V  F  K

                .         .         .         .         .         .
12001  GGACTGGCCCGCCGCGCTGCCCCCCGGAGTGGAGCTGCTCACCGCGCACCTGCCGGGACG  12060
        D  W  P  A  A  L  P  P  G  V  E  L  L  T  A  H  L  P  G  R

                .         .         .         .         .         .
12061  CGGCGACCGGTTCACCGAACCGCCCCCGGCCACCCTGGAGGAGACCGCCGAGCGGCTGTG  12120
        G  D  R  F  T  E  P  P  P  A  T  L  E  E  T  A  E  R  L  C

                .         .         .         .         .         .
12121  CGAGGCGCTGCCGCCGAGTGACCTGCCCACGGTCGTCCTCGGCCACAGCATGGGCGCCCT  12180
        E  A  L  P  P  S  D  L  P  T  V  V  L  G  H  S  M  G  A  L

                .         .         .         .         .         .
12181  GCTGGGGTACGAAGTGGCGGCGCGGCTCGCGGCGCGGGGCCGCGCCCCCAACCTGCTGAT  12240
        L  G  Y  E  V  A  A  R  L  A  A  R  G  R  A  P  N  L  L  I

                .         .         .         .         .         .
12241  CGCCGCGGCCTGCCGTCCCCCGCACGTTCCGCCGGACGCCTCCGGTCCGGTGACCGAGGC  12300
        A  A  A  C  R  P  P  H  V  P  P  D  A  S  G  P  V  T  E  A

                .         .         .         .         .         .
12301  CGAGCTGGCGGCCACCCTGCGGGCCGAACGCCCATGGGACACGGCCCTGAGGGACGAGGA  12360
        E  L  A  A  T  L  R  A  E  R  P  W  D  T  A  L  R  D  E  E

                .         .         .         .         .         .
12361  ACTGATGGAAGCGGTGCTGCCCGCCCTGGTCGCCGACATCACGGCCGGCGACCGCTACCA  12420
        L  M  E  A  V  L  P  A  L  V  A  D  I  T  A  G  D  R  Y  H

                .         .         .         .         .         .
12421  CCGCCCGCGGCCCCGCCCGCTCGACCTCCCGCTGAAGGTCTACATCGGCGCCGACGACGA  12480
```

```
        R  P  R  P  R  P  L  D  L  P  L  K  V  Y  I  G  A  D  D  D

                .         .         .         .         .         .
12481  CGGCACCGACTGGCGCACCACCCTGGGCTGGCGCGCGTGCACCGCCCGGGACTGCGAGGT   12540
        G  T  D  W  R  T  T  L  G  W  R  A  C  T  A  R  D  C  E  V

                .         .         .         .         .         .
12541  CGTCGTCCTGCCCGGCGGCCACTACTTCCTGGAGACCGACCGCGCGGCCGTCCTCACCCG   12600
        V  V  L  P  G  G  H  Y  F  L  E  T  D  R  A  A  V  L  T  R

                .         .         .         .         .         .
12601  CGTCGCCACGGACCTCGCCGAAGCCGAGGTAGGGGCATGACCGCGCGCGTCGACGCCACA   12660
        V  A  T  D  L  A  E  A  E  V  G  A  *
                                           M  T  A  R  V  D  A  T
                                           (orf36)
                .         .         .         .         .         .
12661  CCCACCTACCTGGCGGTGCTGGCGGTGCGCGAGGCCCGCGCCCCGCTCCTCGGCAGCTGC   12720
       P  T  Y  L  A  V  L  A  V  R  E  A  R  A  P  L  L  G  S  C

                .         .         .         .         .         .
12721  CTGGCCCGCATGTCCTTCGCGGTGCTGCCGCTCGCCCTGCTGCTGTCGGTCCGGGACGCG   12780
       L  A  R  M  S  F  A  V  L  P  L  A  L  L  L  S  V  R  D  A

                .         .         .         .         .         .
12781  ACGGGGTCGTTCGCCGTCGCCGGACTGACCTCCGGCGCGCTGTCGGCCACGCTCACGCTG   12840
       T  G  S  F  A  V  A  G  L  T  S  G  A  L  S  A  T  L  T  L

                .         .         .         .         .         .
12841  TTCGCGCCCGCCCGCGCCCGGCTGATCGACCGCCGGGGCTCACGGTCCGGACTGGTCCGG   12900
       F  A  P  A  R  A  R  L  I  D  R  R  G  S  R  S  G  L  V  R

                .         .         .         .         .         .
12901  CTGACCGTCCCGTACCTGCTGGGGCTCGCCGTGCTGATCACATTGGCCGAGGCGGAAGCG   12960
       L  T  V  P  Y  L  L  G  L  A  V  L  I  T  L  A  E  A  E  A

                .         .         .         .         .         .
12961  CCCACCGCGGCGCTGCTCGTCGCCGCCGCGGTCGCGGGCGTGTTCGCGCCGCCGCTCGGT   13020
       P  T  A  A  L  L  V  A  A  A  V  A  G  V  F  A  P  P  L  G

                .         .         .         .         .         .
13021  CCGACCATGCGCGTGCTGTGGGCGAGGATCCTGCACGGCCGTCAGCCCCTGCTGCACACC   13080
       P  T  M  R  V  L  W  A  R  I  L  H  G  R  Q  P  L  L  H  T

                .         .         .         .         .         .
13081  GCCTACGCCCTCGACTCCGTCACCGAGGAGGTGGTCTTCACCGTGGGGCCGCTGCTGGCG   13140
       A  Y  A  L  D  S  V  T  E  E  V  V  F  T  V  G  P  L  L  A

                .         .         .         .         .         .
13141  GGCGGCCTGATCGCGGTCGCGGCACCGCTCGCGTCGATGATCACGGTCATGGTGCTGATC   13200
       G  G  L  I  A  V  A  A  P  L  A  S  M  I  T  V  M  V  L  I

                .         .         .         .         .         .
13201  GCGGCCGGTACCGCCTGCTTCGTGCTGTCCGCCGCGACCGCCGCCGCCCCCGCGTCGGGC   13260
       A  A  G  T  A  C  F  V  L  S  A  A  T  A  A  A  P  A  S  G

                .         .         .         .         .         .
13261  GAAGCCGACGAGGACCGGCCGCACGGCCGGCCCATGGCTCTGCCCGGGATGCGCACGATC   13320
       E  A  D  E  D  R  P  H  G  R  P  M  A  L  P  G  M  R  T  I

                .         .         .         .         .         .
13321  GTGCTGTCCTTCGGCGGCGTCGGCCTGGTCGTCGGGGTGCTCCAGGTCGTCCTGCCGTTC   13380
       V  L  S  F  G  G  V  G  L  V  V  G  V  L  Q  V  V  L  P  F

                .         .         .         .         .         .
13381  ATCGCCGACCACGCGGGCTCGCCCGGCGCGGGCGGCATCCTGCTGTCCATGCTGTCGGCG   13440
       I  A  D  H  A  G  S  P  G  A  G  G  I  L  L  S  M  L  S  A

                .         .         .         .         .         .
13441  GGCAGCGCGGTCGGCGGCCTCGCCTACGGGCGGATCGCCTGGCGCTCGACGCCCGTGCGG   13500
       G  S  A  V  G  G  L  A  Y  G  R  I  A  W  R  S  T  P  V  R

                .         .         .         .         .         .
13501  CGGTTCGTGGTGCTCGTCACCGGGTTCACGCTGGCGGTGCTGCCGCTGTGCCTGACCGCG   13560
       R  F  V  V  L  V  T  G  F  T  L  A  V  L  P  L  C  L  T  A

                .         .         .         .         .         .
13561  AGCCCGGTGCCGGCCGGGGCCTTCGCCCTCCTCGTGGGACTCTGCCTCGCCCCGCTGTTC   13620
       S  P  V  P  A  G  A  F  A  L  L  V  G  L  C  L  A  P  L  F

                .         .         .         .         .         .
13621  ACCACCGCCTACCTGCTGGTCAACGACCTGGTGACGGCGTCGGGGACCGCACCCACCGAG   13680
       T  T  A  Y  L  L  V  N  D  L  V  T  A  S  G  T  A  P  T  E
```

```
                 .         .         .         .         .         .
13681   GCCAACACCTGGGTCTCCACGGCCAATAACGGAGGGTTCGCCGCGGGCAGCGCCGCCGCC    13740
        A  N  T  W  V  S  T  A  N  N  G  G  F  A  A  G  S  A  A  A

                 .         .         .         .         .         .
13741   GGTGTGCTGCTCGACTCCCGGGGCCCCACCGTCACCGTCACCGCCGCGTTCGCGGTCGCC    13800
        G  V  L  L  D  S  R  G  P  T  V  T  V  T  A  A  F  A  V  A

                 .         .         .         .         .         .
13801   GCCGCGACCGCCGTCATGACCGTTCTGCGCCGCCGGACCCTGCTCCTCGGCGCCGGACAC    13860
        A  A  T  A  V  M  T  V  L  R  R  R  T  L  L  L  G  A  G  H

                 .         .         .         .         .         .
13861   CCCGAACCGGCCGCCGCCACACCCGCCGACCGCACCGCACCCGCCGAAGCCGAGGAGTGA    13920
        P  E  P  A  A  A  T  P  A  D  R  T  A  P  A  E  A  E  E  *

                 .         .         .         .         .         .
13921   ACCGATCGTGTCCAAGAACGCGGCGCACTGGTCGCGCATCCGCACAGGGGACGCCCCCGG    13980
               M  S  K  N  A  A  H  W  S  R  I  R  T  G  D  A  P  G
               (orf37)
                 .         .         .         .         .         .
13981   CGTCGTACTCGCCGTGGACTTCTACGGAACGGGCCGCCAGGAAGCCACCTTCCGCCACCT    14040
         V  V  L  A  V  D  F  Y  G  T  G  R  Q  E  A  T  F  R  H  L

                 .         .         .         .         .         .
14041   GTGCGACCTGCTCACGGATCCGGTCGAGGTCTGGCACGCGGTCCCGCCCGCCCCGGACGG    14100
         C  D  L  L  T  D  P  V  E  V  W  H  A  V  P  P  A  P  D  G

                 .         .         .         .         .         .
14101   CGACTGGTCCACGGCCACCGGCGCCGGTCACCTGCGCTGGTGGACCGAGGGGCTCGACAC    14160
         D  W  S  T  A  T  G  A  G  H  L  R  W  W  T  E  G  L  D  T

                 .         .         .         .         .         .
14161   GGTCCTCGCGGGACGGCCGGTGCGGGCCCTCGTCGGCTACTGCGCGGGCGGCGTCTTCGC    14220
         V  L  A  G  R  P  V  R  A  L  V  G  Y  C  A  G  G  V  F  A

                 .         .         .         .         .         .
14221   CTCGGCCCTCGCCGACGCCCTCGTCGAACGGGAGGGCCACCGGCCGCGGGTCGTGCTGTT    14280
         S  A  L  A  D  A  L  V  E  R  E  G  H  R  P  R  V  V  L  F

                 .         .         .         .         .         .
14281   CAACCCCAGCGCGCCCGGCGTCGCCACGCTCACCCGCGACTTCCGCGGTCTGATCGCCGG    14340
         N  P  S  A  P  G  V  A  T  L  T  R  D  F  R  G  L  I  A  G

                 .         .         .         .         .         .
14341   CATGGACCTCCTCACGGACGGGGAACGCGCCGCTCTGCTGGCCGAGACGACCGCGATCCG    14400
         M  D  L  L  T  D  G  E  R  A  A  L  L  A  E  T  T  A  I  R

                 .         .         .         .         .         .
14401   GCGGGCACACGCCCCCGACGCGCTGGTACCGGTCGCCGAACGCTACGCCGCCCTGTACCG    14460
         R  A  H  A  P  D  A  L  V  P  V  A  E  R  Y  A  A  L  Y  R

                 .         .         .         .         .         .
14461   CGAGGGCTGCGACCTCCTGTGCGAGCGGCTCGGCGTGGACGCCTCCTTCGGCGCCGAACT    14520
         E  G  C  D  L  L  C  E  R  L  G  V  D  A  S  F  G  A  E  L

                 .         .         .         .         .         .
14521   GGCCGCCGTCCTCCACTCCTACCTGGCCTACCTCACGGCGGCGCTCGACGTGCCCCCCAC    14580
         A  A  V  L  H  S  Y  L  A  Y  L  T  A  A  L  D  V  P  P  T

                 .         .         .         .         .         .
14581   CCCGCTGTGGCGCGGCGCCGTCTCGCTCACCTCCCGCGAGCACCAGGGCACCGACTTCAC    14640
         P  L  W  R  G  A  V  S  L  T  S  R  E  H  Q  G  T  D  F  T

                 .         .         .         .         .         .
14641   CGACGTCGAGCACGGCTTCGACGTCGCCCGTGCCGAACTGCTGAGCTCCCCCCAGGTCGT    14700
         D  V  E  H  G  F  D  V  A  R  A  E  L  L  S  S  P  Q  V  V

                 .         .         .         .         .         .
14701   CGCGGCGCTGACCGCGCTCCTCCGCGAACACGAGGCGAGCCGATGACCCTCACCCTGCGG    14760
         A  A  L  T  A  L  L  R  E  H  E  A  S  R  *
                                                  M  T  L  T  L  R
                                                  (orf38)
                 .         .         .         .         .         .
14761   GACGCCTTCCTCGACCAGGCCGCCCGGACCCCCGACGCCCACGCCGTCGTACACGGCGAC    14820
        D  A  F  L  D  Q  A  A  R  T  P  D  A  H  A  V  V  H  G  D

                 .         .         .         .         .         .
14821   ACTGTATGGACGTACCGCGAACTGGAACTGCGGGCCGGCCGCATGGCCCGGACGCTGGCC    14880
        T  V  W  T  Y  R  E  L  E  L  R  A  G  R  M  A  R  T  L  A
```

```
                  .         .         .         .         .         .
14881   GCACGCGGCGCGGGCCCCGGCACGCTGGTGGCGGTACGCCTGCCGCGCGGTCCCGAACCG   14940
        A  R  G  A  G  P  G  T  L  V  A  V  R  L  P  R  G  P  E  P

                  .         .         .         .         .         .
14941   GTCGCCGCGCTCCTCGCGGTCGTGCTGACGGGAGCGGGCTACGTGCCGCTCGCCGACGAC   15000
        V  A  A  L  L  A  V  V  L  T  G  A  G  Y  V  P  L  A  D  D

                  .         .         .         .         .         .
15001   GACCCGCCGGACCGGTGCCGGCACATCCTCGACGACTGCGCCGCCGCGCTGCTGCTGGCC   15060
        D  P  P  D  R  C  R  H  I  L  D  D  C  A  A  A  L  L  L  A

                  .         .         .         .         .         .
15061   GAGCACCCCTCGCGGGACGGACGCACCCTCACCCCGGACGAGGCGCTGGCACCCGCCCGC   15120
        E  H  P  S  R  D  G  R  T  L  T  P  D  E  A  L  A  P  A  R

                  .         .         .         .         .         .
15121   CCGTTCGACGCGGCCCCGGTGCGGGCCGGCGACCCGGCGTACGTGATCTACACCTCCGGC   15180
        P  F  D  A  A  P  V  R  A  G  D  P  A  Y  V  I  Y  T  S  G

                  .         .         .         .         .         .
15181   TCCAGTGGCCGTCCGAAGGGCGTGCTGGTCGAACAGGGCGCGCTCGGCGCCTACCTGGCA   15240
        S  S  G  R  P  K  G  V  L  V  E  Q  G  A  L  G  A  Y  L  A

                  .         .         .         .         .         .
15241   CAGGCCCGCGCGCGCTACGACGGGCTGTCCGGACGGACGGTGCTGCACTCCTCGCTGTCC   15300
        Q  A  R  A  R  Y  D  G  L  S  G  R  T  V  L  H  S  S  L  S

                  .         .         .         .         .         .
15301   TTCGACATGGCCGTGACCAGTCTGTGGGGCCCGCTCGTCAGCGGCGGCGCGATCCACGTG   15360
        F  D  M  A  V  T  S  L  W  G  P  L  V  S  G  G  A  I  H  V

                  .         .         .         .         .         .
15361   CTCGACCTGAAGGCGATCGCCTCCGGCACCCAGCCGCCGCCCGCCGCCTCGGCACGTCCG   15420
        L  D  L  K  A  I  A  S  G  T  Q  P  P  P  A  A  S  A  R  P

                  .         .         .         .         .         .
15421   TCCTTCCTCAAGGTCACTCCGTCCCACCTGCCGCTGCTGGGCCTGCTGCCGGACTCCTGC   15480
        S  F  L  K  V  T  P  S  H  L  P  L  L  G  L  L  P  D  S  C

                  .         .         .         .         .         .
15481   CTGCCCACCGGGCAACTCGTGATCGGCGGCGAGGCGCTGACCGGCTCCGCGCTCGGACCC   15540
        L  P  T  G  Q  L  V  I  G  G  E  A  L  T  G  S  A  L  G  P

                  .         .         .         .         .         .
15541   TGGCGCGCCGCGCACCCCGACGTCACGGTCGTCAACGAGTACGGGCCCACCGAGGCGACC   15600
        W  R  A  A  H  P  D  V  T  V  V  N  E  Y  G  P  T  E  A  T

                  .         .         .         .         .         .
15601   GTCGGCTGCTGCGCGTACACCGTCCGCCCCGGTGACGCCGTGGACCCGGGTGCCGTCCCC   15660
        V  G  C  C  A  Y  T  V  R  P  G  D  A  V  D  P  G  A  V  P

                  .         .         .         .         .         .
15661   ATCGGACGGCCGTTCGCGGGCACCCGCCTGTACGTGCTCGACGCGGACGGCGAGCCGGTC   15720
        I  G  R  P  F  A  G  T  R  L  Y  V  L  D  A  D  G  E  P  V

                  .         .         .         .         .         .
15721   GCCGTGGGCGGTGTGGGTGAACTGCACATCGCGGGCGACCAGTTGGCGCGCGGATACCTG   15780
        A  V  G  G  V  G  E  L  H  I  A  G  D  Q  L  A  R  G  Y  L

                  .         .         .         .         .         .
15781   GGGCGCCCGCGGCTGACCGAGGAACGCTTCGTCCCGGACCCGTTCGCCGCCGACGGCTCC   15840
        G  R  P  R  L  T  E  E  R  F  V  P  D  P  F  A  A  D  G  S

                  .         .         .         .         .         .
15841   CGGATGTACCGCACCGGCGACCTGGTGCGCGAACGCCCGGACGGCGACCTGGAGTACCTC   15900
        R  M  Y  R  T  G  D  L  V  R  E  R  P  D  G  D  L  E  Y  L

                  .         .         .         .         .         .
15901   GGGCGCGCGGACGGGCAGGTGAAGGTCTCCGGGTACCGGATCGAGCCCGGCGAGATCGAG   15960
        G  R  A  D  G  Q  V  K  V  S  G  Y  R  I  E  P  G  E  I  E

                  .         .         .         .         .         .
15961   GCCGTGCTCCGCGGCCACGCGGGGGTGAGGGACTGCGCGGTCGTCGCCGTCGGCGAGGCG   16020
        A  V  L  R  G  H  A  G  V  R  D  C  A  V  V  A  V  G  E  A

                  .         .         .         .         .         .
16021   GACGCCCGCCGGCTCGTCGCCTACGTGGTACCGGACCCGGACTCCCCGCCCGGCACCGCC   16080
        D  A  R  R  L  V  A  Y  V  V  P  D  P  D  S  P  P  G  T  A

                  .         .         .         .         .         .
16081   GCGCCGGCGCGGCACGCGGCCGAGGCGCTGCCGCCGTACATGGTGCCGGCGACGTTCGTC   16140
```

```
      A  P  A  R  H  A  A  E  A  L  P  P  Y  M  V  P  A  T  F  V

               .         .         .         .         .         .
16141 ACCGTGCCCGAACTGCCGCTCACCCCCAACGGGAAGCTCGACCGGGACGCGCTGCCCGGC 16200
      T  V  P  E  L  P  L  T  P  N  G  K  L  D  R  D  A  L  P  G

               .         .         .         .         .         .
16201 CCCCCTGCCGGCGACGCCGGGCCGGGCGACCGCACCCCGGCCGAGACCCTGCTGTGCGAG 16260
      P  P  A  G  D  A  G  P  G  D  R  T  P  A  E  T  L  L  C  E

               .         .         .         .         .         .
16261 CTGCTGGCACGGGCCCTGGGCATCCCGGAGATCGACGCCGACGCCGACTTCCTGACGTCC 16320
      L  L  A  R  A  L  G  I  P  E  I  D  A  D  A  D  F  L  T  S

               .         .         .         .         .         .
16321 GGCGGCACCAGCATCACCGCGCTGAAGCTGGTCGCCGGCGCCCGCCGGGTCGGCATCCGC 16380
      G  G  T  S  I  T  A  L  K  L  V  A  G  A  R  R  V  G  I  R

               .         .         .         .         .         .
16381 CTCGAACTCACCACCGTCCTGCGCGAACGCACGGTGCGCCGCATCCTGGCGGCCCAGCCC 16440
      L  E  L  T  T  V  L  R  E  R  T  V  R  R  I  L  A  A  Q  P

               .         .         .         .         .         .
16441 GACGCCGCCTCGCCCCTCGCCGAAGGAGTGCCCGAGTGACCGGTTCCGTAACGCTCACCC 16500
                                         M  T  G  S  V  T  L  T  P
      D  A  A  S  P  L  A  E  G  V  P  E  *   (orf39)

               .         .         .         .         .         .
16501 CCCTCGGCGGGATCATCCCCAGGCCCCGCGGCGAGGGGCTCACCACCGGCGCCGAGTACG 16560
       L  G  G  I  I  P  R  P  R  G  E  G  L  T  T  G  A  E  Y  D

               .         .         .         .         .         .
16561 ACCTGGGGCCGCTCGGCGACGCGGGCCCCGACTGgGTGCGGGCCCACGGCCCGCGACTGC 16620
       L  G  P  L  G  D  A  G  P  D  W  V  R  A  H  G  P  R  L  R

               .         .         .         .         .         .
16621 GCGAGCGCCTCGCCACCGACGGGCTGATCCTGCTGCACGGTCTGCCCACCGACGGAGACG 16680
       E  R  L  A  T  D  G  L  I  L  L  H  G  L  P  T  D  G  D  G

               .         .         .         .         .         .
16681 GCGTCGACGGCTTCCACGACGTCGTCGGCTCCGTCGGCGGCGACCCGCTGCCCTACACCG 16740
       V  D  G  F  H  D  V  V  G  S  V  G  G  D  P  L  P  Y  T  E

               .         .         .         .         .         .
16741 AGCGCTCCACCCCGCGCAGCGTGGTCAAGGGCAACATCTACACCTCGACCGAGTACCCGG 16800
       R  S  T  P  R  S  V  V  K  G  N  I  Y  T  S  T  E  Y  P  A

               .         .         .         .         .         .
16801 CCGACCAGCCCATCCCGATGCACAACGAGAACTCCTACGCCGCCCATTGGCCGTCCACGC 16860
       D  Q  P  I  P  M  H  N  E  N  S  Y  A  A  H  W  P  S  T  L

               .         .         .         .         .         .
16861 TCTACTTCTTCTGCCACACCGCGCCGGACACCGGCGGGGCCACGCCGATCGCCGACGGCC 16920
       Y  F  F  C  H  T  A  P  D  T  G  G  A  T  P  I  A  D  G  R

               .         .         .         .         .         .
16921 GCGCCGTCCTCGACCTCATCCCGGCCGAGGTCAGGCGGCGGTTCTCCCAAGGGGTCGTCT 16980
       A  V  L  D  L  I  P  A  E  V  R  R  R  F  S  Q  G  V  V  Y

               .         .         .         .         .         .
16981 ACACCCGTACGTTCCGCGCCGACATGGGACTGAGCTGGCAGGAAGCGTTCCAGACCGAGG 17040
       T  R  T  F  R  A  D  M  G  L  S  W  Q  E  A  F  Q  T  E  D

               .         .         .         .         .         .
17041 ACCGCGGCGACGTCGAACGCCATTGCCGCGCCCACGGCCAGGAGTTCTCCTGGGACGGCG 17100
       R  G  D  V  E  R  H  C  R  A  H  G  Q  E  F  S  W  D  G  D

               .         .         .         .         .         .
17101 ACGTCCTGCGCACCCGCCACCACCGCCCGGCGACCGCCGTCGACCCCGGCACCGGAGCCG 17160
       V  L  R  T  R  H  H  R  P  A  T  A  V  D  P  G  T  G  A  E

               .         .         .         .         .         .
17161 AGGTGTGGTTCAACCAGGCGCACCTGTTCCACCCGTCCAGCCTGGATCCCGACCTGCGCC 17220
       V  W  F  N  Q  A  H  L  F  H  P  S  S  L  D  P  D  L  R  Q

               .         .         .         .         .         .
17221 AGGTGCTCCTGGAGACGTACGGCGAGAACGGCCTGCCCCGCGACGCCCTGTTCGCCGACG 17280
       V  L  L  E  T  Y  G  E  N  G  L  P  R  D  A  L  F  A  D  G

               .         .         .         .         .         .
17281 GCACCCCGATCCCCGACGCCGACCTGGCAACGGTCCGCGCGGCCTACACCCGCGCCGCGC 17340
       T  P  I  P  D  A  D  L  A  T  V  R  A  A  Y  T  R  A  A  L
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                 .         .         .         .         .         .
17341   TCGCGCTGCCGTGGCGAGAGGGCGACATCATGCTGGTCGACAACCTGAGGATGGCCCACG   17400
         A  L  P  W  R  E  G  D  I  M  L  V  D  N  L  R  M  A  H  G

                 .         .         .         .         .         .
17401   GCCGCGAGCCCTTCACCGGCGAGCGCCGCGTACTCGTCGCGATGACCTCGGCGGACTCAT   17460
         R  E  P  F  T  G  E  R  R  V  L  V  A  M  T  S  A  D  S  *

                 .         .         .         .         .         .
17461   GAGCCGTGCCGACGCATCGGCACGCCGTCCTCCCGTCGGGGCGCTACCATCGCCGCTGTC   17520

                 .         .         .         .         .         .
17521   TCGGCCATCACCCCACCCGGGCGGAGGCAACCGGCCGTGCACATCCCCGCCGTGGTCGCC   17580

                 .         .         .         .         .         .
17581   ACGGCACGCGCGATCACCCGCGCCATGACCGCCCAGCCCGTTGTCACATCTGCGGAGGCG   17640

                 .         .         .         .         .         .
17641   CCGCGATGACAGAGGTCCGAGGTGAACTGATCCGGGCGCTGCCGGGTGTGCTGGAGGCGC   17700
            M  T  E  V  R  G  E  L  I  R  A  L  P  G  V  L  E  A  R
            (orf40)
                 .         .         .         .         .         .
17701   GTGCGGCGCGGGCGGGGCACACGACCGCCTTCCTCGACGCACGACGGTGTGTCACGTACC   17760
         A  A  R  A  G  H  T  T  A  F  L  D  A  R  R  C  V  T  Y  R

                 .         .         .         .         .         .
17761   GGGAGTTGGAGGCGCGCACCCGCCGGCTGGCGGGGTCACCTGGTGCGGTTGGGGGTGCGC   17820
         E  L  E  A  R  T  R  R  L  A  G  S  P  G  A  V  G  G  A  Q

                 .         .         .         .         .         .
17821   AGGGGCAGACCGGGTGGCGCTCGTCAATGGGCAACCGGGGTGGAGATGGCGGAGGGTTCC   17880
         G  Q  T  G  W  R  S  S  M  G  N  R  G  G  D  G  G  G  F  P

                 .         .         .         .         .         .
17881   CTCCCCGGTGCTGCGGGCCGGAGCGGTAGGGGTGCCGCTCGATTCCGGGGCCACGGACGC   17940
         P  R  C  C  G  P  E  R  *  G  C  R  S  I  P  G  P  R  T  R

                 .         .         .         .         .         .
17941   GGAGCTCGCGTACTTCCTCGACGACTGTGGAGCGGTGGCGGTGGTCACCGAGGAGACGCT   18000
         S  S  R  T  S  S  T  T  V  E  R  W  R  W  S  P  R  R  R  C

                 .         .         .         .         .         .
18001   GCTGCCGCGGGTCTCGCGATCGGCGGGCGTACGGATCCTGGTGGGGGGTTCGGACGCCGT   18060
         C  R  G  S  R  D  R  R  A  Y  G  S  W  W  G  V  R  T  P  S

                 .         .         .         .         .         .
18061   CCCGGAGGGAGCGGCTGCCGGCATCCACTCCTTCGAGCGGCTCGCGGCGTCGGATCCGGG   18120
         R  R  E  R  L  P  A  S  T  P  S  S  G  S  R  R  R  I  R  G

                 .         .         .         .         .         .
18121   GTGCGCGCCACGGGACGACCTCGGCCTCGACGAGCCGGCCTGGATCCTCTACACGTCGGG   18180
         A  R  H  G  T  T  S  A  S  T  S  R  P  G  S  S  T  R  R  G

                 .         .         .         .         .         .
18181   GACCACGGGCCGGAGCAAGGGCGTGGTCTGCGGCCAGCGCGCCGCGCTGTGGTCCGTGGC   18240
         P  R  A  G  A  R  A  W  S  A  A  S  A  P  R  C  G  P  W  R

                 .         .         .         .         .         .
18241   GGCGGCGTACGTGCCGTCGTGGGGTCTGGGGCCGCAGGACCGGCTGTTGTGGCCGCTGCC   18300
         R  R  T  C  R  R  G  V  W  G  R  R  T  G  C  C  G  R  C  P

                 .         .         .         .         .         .
18301   CATGTTCCACGCCTACGCGCACTCGCTGTGCCTGCTCGGGGTGGTGGCCGTGGGCGCGAG   18360
         C  S  T  P  T  R  T  R  C  A  C  S  G  W  W  P  W  A  R  A

                 .         .         .         .         .         .
18361   CGCGTACCTCCTCGACCGGGGCGCGAGCGTCGTCCGGGCGCTTGAGGAACAGCGGTGCAG   18420
         R  T  S  S  T  G  A  R  A  S  S  G  R  L  R  N  S  G  A  A

                 .         .         .         .         .         .
18421   CGTCGTGGCCGGTGTACCCGCCACCTACCGCCTGCTCACGAGCGCCTTCCGCGACGCCCC   18480
         S  W  P  V  Y  P  P  P  T  A  C  S  R  A  P  S  A  T  P  P

                 .         .         .         .         .         .
18481   CCGGCCACCGGCCGGCCTGCGACTGTGCGTCACCGGGGGCTGCGCCGTGCCCGCCGGGGC   18540
         G  H  R  P  A  C  D  C  A  S  P  G  A  A  P  C  P  P  G  L

                 .         .         .         .         .         .
18541   TGCGGGCGGACGTTGAGGAGCTGCTGGGCGTCCCGCTGCTCGACGGTTACGGCAGTACCG   18600
         R  A  D  V  E  E  L  L  G  V  P  L  L  D  G  Y  G  S  T  E
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                 .         .         .         .         .         .
18601   AGACCTGCGGCAAGATCACGGTTGAGCGGCTCGGCGGCTCCCGGGAGGGCGGTTGCCGGG   18660
           T  C  G  K  I  T  V  E  R  L  G  G  S  R  E  G  G  C  R
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SEQ ID NO: 3 BLM gene PPTase ORFS 41

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                 .         .         .         .         .         .         .         .
    1   GGATCCTGCGCTACCCGGACTTCGCCCAGTGGTGCGGCACCGAGCTCACCGCCGACTGGCACGTCCGCTTCCGGGCCGCC   80
                 .         .         .         .         .         .         .         .
   81   GCCGCGGTCTACGGGCATCTGCACATCCCCCGCGTGACCCGGTACGACGGCGTCCGCTTCGAGGAGGTGTCGGTCGGCTA   160
                 .         .         .         .         .         .         .         .
  161   CCCGCGCGAGTGGCGGCCCCGGCCGCCCCGCGAGCCGCTCCGGCAGATCCTGCCCCAGCCCGTCGACGAGCCGGGAGCCC   240
                 .         .         .         .         .         .         .         .
  241   TCTGGTGATCGCCGCCCTCCTGCCCTCCTGGGCCGTCACCGAACACGCCTTCACCGACGCCCCGGACGACCCGGTGAGCC   320
    1        M  I  A  A  L  L  P  S  W  A  V  T  E  H  A  F  T  D  A  P  D  D  P  V  S  L  26
                 .         .         .         .         .         .         .         .
  321   TCCTCTTCCCCGAGGAGGCCGCCCACGTCGCCCGCGCCGTCCCCAAGCGCCTGCACGAGTTCGCCACCGTCCGGGTGTGC   400
   27      L  F  P  E  E  A  A  H  V  A  R  A  V  P  K  R  L  H  E  F  A  T  V  R  V  C    52
                 .         .         .         .         .         .         .         .
  401   GCCCGCGCCGCCCTCGGCCGGCTGGGCCTCCCGCCCGGTCCGCTGCTGCCCGGCCGACGGGGCGCGCCGAGCTGGCCGGA   480
   53    A  R  A  A  L  G  R  L  G  L  P  P  G  P  L  L  P  G  R  R  G  A  P  S  W  P  D   79
                 .         .         .         .         .         .         .         .
  481   CGGGGTGGTGGGGAGCATGACGCACTGTCAGGGCTTCCGGGGCGCCGCGGTCGCCCGGGCCGCCGACGCCGCGTCGCTCG   560
   80     G  V  V  G  S  M  T  H  C  Q  G  F  R  G  A  A  V  A  R  A  A  D  A  A  S  L  G  106
                 .         .         .         .         .         .         .         .
  561   GGATAGACGCCGAGCCGAACGGGCCGCTCCCGGACGGCGTCCTCGCCATGGTCTCGCTGCCGTCCGAGCGCGAGTGGCTC   640
  107      I  D  A  E  P  N  G  P  L  P  D  G  V  L  A  M  V  S  L  P  S  E  R  E  W  L    132
                 .         .         .         .         .         .         .         .
  641   GCCGGACTGGCGGCCCGCCGGCCGGACGTGCACTGGGACCGGCTGCTGTTCAGCGCCAAGGAGAGCGTCTTCAAGGCGTG   720
  133    A  G  L  A  A  R  R  P  D  V  H  W  D  R  L  L  F  S  A  K  E  S  V  F  K  A  W   159
                 .         .         .         .         .         .         .         .
  721   GTACCCGCTGACCGGCCTGGAGCTGGACTTCGACGAGGCCGAGCTGGCCGTCGATCCGGACGCCGGGACGTTCACGGCCC   800
  160     Y  P  L  T  G  L  E  L  D  F  D  E  A  E  L  A  V  D  P  D  A  G  T  F  T  A  R  186
                 .         .         .         .         .         .         .         .
  801   GGCTGCTGGTGCCGGGACCGGTGGTCGGCGGCCGTCGGCTGGACGGGTTCGAGGGGCGCTGGGCGGCGGGCGAGGGCCTC   880
  187      L  L  V  P  G  P  V  V  G  G  R  R  L  D  G  F  E  G  R  W  A  A  G  E  G  L    212
                 .         .         .         .         .         .         .         .
  881   GTCGTCACGGCCATCGCCGTCGCGGCGCCGGCCGGTACCGCGGAGGAATCGGCGGAAGGGGCCGGGAAGGAAGCGACTGC   960
  213    V  V  T  A  I  A  V  A  A  P  A  G  T  A  E  E  S  A  E  G  A  G  K  E  A  T  A   239
                 .         .         .         .         .         .         .         .
  961   GGACGACCGGACCGCCGTCCCGTAAACCGCCCCGAACACCGGCGTGGCGCCCGCCGACCGTGTCGGGGGCGCCACGAACG   1040
  240     D  D  R  T  A  V  P  *                                                           247
                 .         .         .         .         .         .         .         .
 1041   GGCGCCGGCCCGGCGGGCCCTCCGCCGTGCGGAGCGGAGGCCCGGCGCGGACGCGCCCGGTGTCGTCGGATACGTGCGTC   1120
                 .         .         .         .         .         .         .         .
 1121   AGTCGGCGACGCAGACGTTGCCGTTGGTCGAGTTGAGCAGCCCGACGATGTCGATGGTGTTGCCGCAGAGGTTGATGGGG   1200
                 .         .         .         .         .         .         .         .
 1201   ATGTGGACGGGGATCTGGATGACGTTGCCCGAGACGACGCCCGGGGAGCCGACGGCCGCCCCCTTGGCGTTCGAGTCGGC   1280
                 .         .         .         .         .         .         .         .
 1281   GAGGGCGGTGCCGGAGACGCCGGCGAGCGCCGTGCCCACGGTGGCGGTGAGGGCCGCTGCCTTGGCGATTCGTGACATGG   1360
                 .         .         .         .         .         .         .         .
 1361   GGTGACACCTTCGTTCGGTCTGACAGGGTCGAGCTCACGGCCTCTGACGGCCGGGAGCCCGGATCAACGCCCGATCACCC   1440
                 .         .         .         .         .         .         .         .
 1441   CGAAGGTTTCGAATCGTGCGGCGGACGGGTGACCGGCGGCCGAACGGCCTCGCCGGGCCCCCGGAAGGTGCCATGACGTC   1520
                 .         .         .         .         .         .         .         .
 1521   CGTGCGCCATCTGTACAGCCCGGTCCCGCGCCGCGTACAAGGGACGGACGGACGGCCGGTGGACGGACGACCGGCGGGGA   1600
                 .         .         .         .         .         .         .         .
 1601   GGGGAGGCCATGAGCCGGATCGCGATCGTCGGGGCGGGTCAGGCCGGACTGCATCTGGCGCTGGGGCTGCTGGGGGCGGG   1680
                 .         .         .         .         .         .         .         .
 1681   GAGCGGCTCTTCCCGTCACGAGGTGCTGCTCGTGTCCGACGGGACGCCGGACGAGATCCGCGCCGGGCGGGTGCGGTCGA   1760

 1761   C  1761
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